

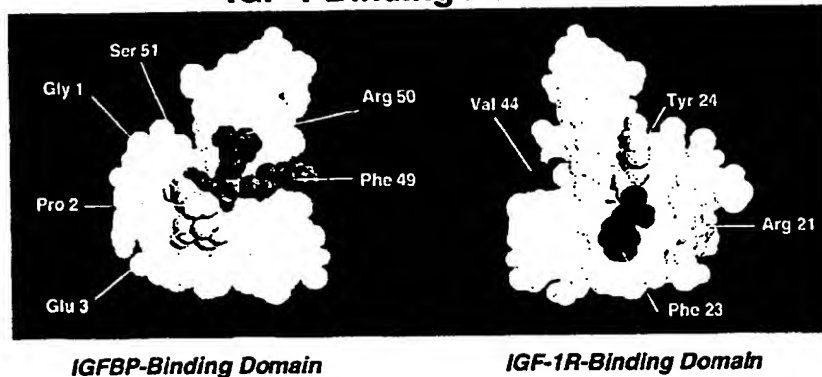


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(54) Title: FRAGMENTS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN AND INSULIN-LIKE GROWTH FACTOR, AND USES THEREOF

IGF-1 Binding Domains

**(57) Abstract**

The present invention provides the portions of IGFBP and IGF peptides which account for IGF-IGFBP binding. Thus, the present invention provides an isolated IGF binding domain of an IGFBP or modifications thereof, which binds IGF with at least about the same binding affinity as the full length IGFBP. The present invention also provides an IGF antagonist that reduces binding of IGF to an IGF receptor. Further provided is a fragment of IGF or modification thereof, wherein the fragment or modification thereof binds to a binding domain of IGFBP. The present invention further provides a protein complex, comprising the IGF binding domain of IGFBP of the invention and the IGF fragment of the invention. The present invention provides methods of treating a subject with cancer and of preventing cancer in a subject, of treating a subject with a diabetic complication exacerbated by IGF and of preventing diabetic complications exacerbated by IGF comprising administering to the subject the IGF antagonist of the present invention. Also provided is a method of treating a subject with an ischemic injury or preventing an ischemic injury in a subject, comprising administering to the subject the IGF fragment or IGFBP antagonist of the invention.

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FRAGMENTS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN AND INSULIN-LIKE GROWTH FACTOR, AND USES THEREOF

This application claims priority to U.S. provisional application Serial No.
5 60/104,528 filed on October 16, 1998. The 60/104,528 provisional patent application
is herein incorporated by this reference in its entirety. This invention was made with
government support under the National Institutes of Health, National Cancer Institute
grant number CA 78887. The government has certain rights in this invention.

10

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to fragments of insulin-like growth factor binding
protein (IGFBP) and, specifically, the insulin-like growth factor (IGF) binding domain
15 of IGFBP. The present invention also relates to fragments of IGF and, specifically, the
IGFBP binding domain on IGF. The present invention also relates to the uses of the
fragments as therapeutic agents in the medical field.

Background Art

20 Insulin-like growth factors (IGFs), peptides which are structurally similar to
insulin, have been implicated in a variety of cellular functions and disease processes.
Thus, IGF has been suggested as a therapeutic tool in a variety of diseases and injuries
(for review, see Lowe, Scientific American March/April 1996, p. 62). The IGF-1
receptor (IGF-1R) has been shown to bind both IGF-1 and IGF-2, homologous proteins
25 which are 70 and 67 amino acids in length, respectively (*See* Humbel, 1990). The IGF-
1R binds IGF-1 and IGF-2 with high affinity and insulin with lower affinity.

IGF-1R plays a central role in normal cell growth and development (*See* Daughaday, 1989). There is mounting evidence, however, that IGF-1R signaling also plays an essential role in tumor cell growth, as well as cell transformation and tumorigenesis (*See* Baserga, 1995). Key examples include loss of metastatic phenotype
5 of murine carcinoma cells by treatment with antisense RNA to the IGF-1R (*See* Long, 1995) and the *in vitro* inhibition of human melanoma cell motility (*See* Stracke, 1989) and of human breast cancer cell growth by the addition of IGF-1R antibodies (*See* Rohlik, 1987). The IGFs are potent breast cancer cell mitogens based on the observation of IGF-1 enhanced breast cancer cell proliferation *in vitro* (*See* Cullen,
10 1990). Breast cancers express IGF-2 and the IGF-1 receptor (IGF-1R), providing all the required effectors for an autocrine-loop based proliferation paradigm (*See* Quinn, 1996; Steller, 1996). Because breast cancer is a common malignancy affecting approximately one in every seven women and is the leading cause of death from cancer in North American women (*See* LeRoith, 1995), new, rational therapies are required for
15 intervention. Because IGF-1 can suppress apoptosis, cells lacking IGF-1Rs or having compromised IGF-1R signaling pathways may give rise to tumor cells that selectively die via apoptosis (*See* Long, 1995). Furthermore, it has recently become evident that alterations in IGF signaling in the context of other disease states such as diabetes, may be responsible for exacerbating the complications of retinopathy (Smith et al., 1997)
20 and nephropathy (Horney et al., 1998).

The IGF binding proteins (IGFBPs) modulate access of the IGFs to the IGF-1R. IGFBPs are a family of at least 6 soluble proteins that regulate the concentrations of IGF-1 and IGF-2 in the circulation and at the level of the tissue IGF-1R (*See*
25 Clemmons, 1993). In most cases, addition of exogenous IGFBP blunts the effects of IGF-1. For example, the growth stimulating effect of estradiol on the MCF-7 human breast cancer cells is associated with decreased IGFBP-3 mRNA and protein accumulation, while the antiestrogen ICI 182780 causes growth inhibition and increased IGFBP-3 mRNA and protein levels (*See* Huynh, 1996; Oh, 1995). It has also
30 been reported that the *in vitro* inhibition of breast cancer cell proliferation by retinoic

acid may involve altered IGFBP secretion by tumor cells or decreased circulating IGF-1 levels *in vivo* (See LeRoith, 1995; Oh, 1995). Contrary to this finding, treatment of MCF-7 cells with the anti-estrogen tamoxifen, decreases IGF-1R signaling in a manner that was unrelated to decreased IGFBP production (See Lee, 1997). Additional support
5 for the general anti-proliferative effects of the IGFBPs is the striking finding that IGFBP-3 is a target gene of the tumor suppressor, p53 (See Buckbinder, 1995). This suggests that the suppressor activity of p53 is, in part, mediated by IGFBP-3 production and the consequential blockade of IGF action (See Buckbinder, 1995). These results indicate that the IGFBPs can block cell proliferation by modulating paracrine/autocrine
10 processes regulated by IGF-1/IGF-2. A corollary to these observations is the finding that Prostate-Specific Antigen is an IGFBP-3-protease, which upon activation, increases the sensitivity of tumor cells to the actions of IGF-1/IGF-2 due to the proteolytic inactivation of IGFBP-3 (See Cohen, 1994).

15 The IGFBPs complex with IGF-1/IGF-2 and interfere with the access of IGF-1/IGF-2 to IGF-1Rs (See Clemmons, 1993). IGFBP-1, 2 and 3 inhibit cell growth following addition to cells *in vitro* (See Lee, 1997; Feyen, 1991). Further, IGFBP-1 (See McGuire, 1992; Figueroa, 1993), IGFBP-3 (See Oh, 1995; Pratt, 1994) and IGFBP-2 have all been shown to inhibit IGF-1 or estrogen induced breast cancer cell
20 proliferation at nanomolar concentrations *in vitro*. These findings support the idea that the IGFBPs are potent antagonists of IGF action. There is also evidence for a direct effect of IGFBP-3 on cells through its own cell surface receptor, independent of IGF interactions (See Oh, 1993; Valentinis, 1995). Taken together, these findings underscore the importance of IGF and IGF-1R as important targets for therapeutic use.

25

Exploitation of the interaction between IGF and IGFBP in screening, preventing, or treating disease has been limited, however, because of a lack of specific antagonists. To date, only one publication describing the application of an IGF-1/IGF-2 antagonist as a potential therapeutic adjunct in the treatment of cancer exists (See

Pietrzkowski et al., 1992). In that report, a peptide corresponding to the D-region of IGF-1 was synthesized for use as an IGF-1/2 antagonist. This peptide exhibited questionable inhibitory activity against IGF-1. The basis for the observed inhibition is unclear as the D-region does not play a significant role in IGF-1R binding but rather, in IGF-1 binding to the insulin receptor (See Cooke, 1991; Bayne, 1988; Yee, 1994. IGF antagonists whose mechanism of action is via blockade of interactions at the IGF-1R interface may also significantly alter insulin action at the insulin receptor, a disadvantage of such antagonists.

10 Generation of specific antagonists has been restricted, at least in part, because of difficulties in studying the structure of IGF and IGFBP. Due to the inability to obtain crystals of IGF-1 suitable for diffraction studies, for example, an extrapolation of IGF-1 structure based on the crystal structure of porcine insulin was the most important structural road map for IGF-1 available (See Blundell, 1978). Based on studies of
15 chemically modified and mutated IGF-1, a number of common residues between IGF-1 and insulin have been identified as being part of the IGF-1R-insulin receptor contact site, in particular the aromatic residues at positions 23-25. Using NMR and restrained molecular dynamics, the solution structure of IGF-1 was recently reported (See Cooke, 1991). The resulting minimized structure was shown to better fit the experimental
20 findings on modified IGF-1, as well as the extrapolations made from the structure-activity studies of insulin. Prior to the present invention, a specific site on the IGFBPs responsible for IGF binding had not been identified. Furthermore, neither unique antagonists of the IGFs applicable to the treatment of cancer and of diabetic nephropathy and retinopathy nor isolated monobiotinylated IGFs that bind IGFBP with
25 high affinity have been identified prior to the present invention.

SUMMARY OF THE INVENTION

The present invention provides the portions of the IGFBP and IGF peptides which account for IGF-IGFBP binding. Thus, the present invention provides an isolated IGF binding domain on an IGFBP or modifications thereof, which binds IGF with at least about the same binding affinity as the full length IGFBP. The present invention also provides an IGF antagonist, comprising the isolated binding domain of the invention or modification or fragment thereof or comprising a related peptide of 250 or fewer amino acids which reduces binding of IGF to an IGF receptor. Further provided is a fragment of IGF or modification thereof, wherein the fragment or modification thereof binds to a binding domain of IGFBP.

The present invention further provides a protein complex, comprising the IGF binding domain of IGFBP of the invention and the IGF fragment of the invention. The present invention also provides a protein complex, comprising the IGF binding domain of IGFBP and a photoaffinity derivative of an IGF.

Therapeutic uses of the IGFBP and IGF peptides are also provided. Thus, the present invention provides methods of treating a subject with cancer and of preventing cancer in a subject, comprising administering to the subject the IGF antagonist of the invention. The present invention further provides methods of treating a subject with a diabetic complication exacerbated by IGF and of preventing diabetic complications exacerbated by IGF, comprising administering to the subject the IGF antagonist of the invention. Further provided are methods of treating a subject with an ischemic injury or preventing the ischemic injury in a subject, comprising administering to the subject the IGF fragment or IGFBP antagonist of the invention, thereby reducing the ischemic injury in the subject.

The invention also provides an isolated monobiotinylated IGF, wherein the biotinylated IGF has a high affinity for IGFBP. Preferably, the affinity of the biotinylated IGF for IGFBP is comparable to the affinity of unmodified IGF (i.e., having a K_D about 50-300 pM in a competition binding assay). Such biotinylated IGF is useful in therapeutic assays for IGFBP and in screening for IGFBP-mimetics (such as, IGF antagonists).

It is an object of this invention to provide peptides that can serve as stable, protease resistant analogs of the IGFBPs (i.e., IGFBP-mimetics). The IGFBP analogs act as IGF antagonists. Thus, the IGF-1 antagonists of the present invention use the IGF binding site of IGFBPs and thereby avoid the insulin effects of IGF antagonists that act by blockade of interactions at the IGF-1R interface, since blockade of the IGF-1R may significantly alter insulin action at the insulin receptor. The IGFBPs, in contrast, are selective for the IGFs, thus eliminating the possibility of compromising insulin action. The IGF binding site of IGFBP and related antagonists are therapeutically useful in the treatment of cancer and diabetic complications.

Another object of the invention is to provide peptides comprising a fragment of IGF that binds to IGFBP, wherein the fragment decreases IGFBP binding of IGF, thereby increasing the IGF available for binding to the IGF receptor. Such fragments are useful in a variety of disorders and injuries.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the IGFBP-binding site on IGF-1. The N-terminal residues Gly 1, Pro 2 and Glu 3 combine with residues Phe 49, Arg 50 and Ser 51 to form the IGFBP binding domain (left). This association is based on the minimized structure of IGF-1 obtained by NMR and restrained molecular dynamics (*See Cascieri, 1989*). The Gly 1 α -NH₂ group is the site chosen for derivatization with 4-azidobenzoyl-*N*-hydroxysuccinimide ester. Turning the molecule 180° reveals the IGF-1R binding domain on IGF-1 (right). Image obtained in RasMol v 2.6 using coordinates (*See Cascieri, 1989*) from the Brookhaven National Laboratory Protein Database.

Figure 2 shows the primary structures of insulin, IGF-1 and IGF-2. (*See Szabo et al., 1988 referencing Blundell et al., 1978*). The boxed sequences shown are residues conserved across insulin, IGF-1 and IGF-2. Shadowed residues are conserved across all insulins sequenced. Letters at left indicate common regions referred to in text.

Figure 3 shows the pepsin cleavage sites in IGF-1. To verify derivatization of IGF-1 at its N-terminal glycine residue, the purified probe was digested with pepsin (*See Hober et al., 1992*). The rationale behind this approach is that tryptic digestion requires disulfide bond reduction, whereas pepsin digestion does not. Reducing conditions may result in the loss of the azide from the azidobenzoyl moiety. Peak 4 was shown to be monoderivatized, based on the acid-urea gel analysis shown below. The AE fragment highlighted here, contains the N-terminal glycine residue and none of the lysyl residues.

25

Figure 4 shows the primary structures of IGFBPs 1-6. Spatially conserved cysteine residues are shown in gray. The underlined sequence indicates the epitope peptide used to generate anti-IGFBP-2 antibodies. Sequences were aligned using the

Pileup component of the Wisconsin Package version 9.0 (Genetics Computer Group (GCG), Madison, Wisconsin).

Figure 5 shows the tryptic fragments present in the 15.8 kDa fragment. The
5 contiguous peptide sequences identified by MALDI-TOF MS (Matrix Assisted Laser
Desorption Ionization-Time of Flight Mass Spectrometry) are shown in gray. The lines
over the sequence indicate the tryptic fragments detected by MALDI.

Figure 6 shows an IGF binding domain on IGFBP-2, which corresponds to
10 residues 212-287. The bars indicate the locations of disulfide bonds.

Figure 7 shows an IGF binding domain on IGFBP-2, which corresponds to
residues 151-289. The bars indicate the locations of disulfide bonds.

15 Figure 8 shows an IGF binding domain on IGFBP-2, which corresponds to
residues 189-289. The bars indicate the locations of disulfide bonds.

Figure 9 shows an IGF binding domain on IGFBP-2, which corresponds to
residues 226-289. The bars indicate the locations of disulfide bonds.
20

Figure 10 shows an IGF binding domain on IGFBP-2, which corresponds to
residues 226-272. The bars indicate the locations of disulfide bonds.

Figure 11 shows an IGF binding domain on IGFBP-2, which corresponds to
25 residues 232-272. The bars indicate the locations of disulfide bonds.

Figure 12 shows an IGF binding domain on IGFBP-2, which corresponds to a
dimerized motif of residues 232-249 and 236-248. The underlined residues are
repeated in the structure. The bars indicate the locations of disulfide bonds.

Figure 13 shows a dimerized motif of residues 248-270 and 248-272. The underlined residues (248-270) are repeated. The bars indicate the locations of disulfide bonds.

5

Figure 14 shows a construct having the sequence for a 5' segment of IGFBP-2 cDNA, containing the sequence for the signal peptide and N-terminus, and the sequence for the C-terminal fragment of IGFBP.

10 Figure 15 shows a sensorgram that reflects binding of IGFBP-2 (1 µg/10 µl) and IGF-1 biotinylated on residue Lysine-65, which was immobilized on the surface of a sensor chip surface. The gray profile represents an identical injection of analyte into the control flow cell (streptavidin only), providing the signal for non-specific binding and bulk flow effects. The difference between these two signals is the specific binding
15 of IGFBP-2 to IGF-1 (300 response units).

Figure 16 shows a sensorgram that reflects binding of WHKPRPT (SEQ ID NO:76) and IGF-1 biotinylated on residue Lysine-65, which was immobilized on the surface of a sensor chip surface. The gray profile represents an identical injection of
20 analyte into the control flow cell (streptavidin only), providing the signal for non-specific binding and bulk flow effects. The difference between these two signals is the specific binding of WHKPRPT (SEQ ID NO:76) to IGF-1 (15 response units).

Figure 17 shows a sensorgram that reflects binding of WHKPRPR (SEQ ID
25 NO:79) and IGF-1 biotinylated on residue Lysine-65, which was immobilized on the surface of a sensor chip surface. The gray profile represents an identical injection of analyte into the control flow cell (streptavidin only), providing the signal for non-specific binding and bulk flow effects. The difference between these two signals is the specific binding of WHKPRPR (SEQ ID NO:79) to IGF-1 (36 response units).

Figure 18 shows a sensorgram that reflects binding of ERGPLEHLYSLHIPNC (SEQ ID NO:84) and IGF-1 biotinylated on residue Lysine-65, which was immobilized on the surface of a sensor chip surface. The gray profile represents an identical injection of analyte into the control flow cell (streptavidin only), providing the signal for non-specific binding and bulk flow effects. The difference between these two signals is the specific binding of ERGPLEHLYSLHIPNC (SEQ ID NO:84) to IGF-1 (245 response units).

DETAILED DESCRIPTION OF THE INVENTION

10

The present invention provides an isolated peptide comprising an IGF binding domain on an IGFBP or modification thereof, which binds IGF with at least about the same binding affinity as the full length IGFBP. More specifically, the binding domain or modification thereof binds to IGF-1 or IGF-2. By "peptide" is meant a single amino acid sequence, including either a linear sequence or a sequence having an internal disulfide bond, or a peptide complex of two or more amino acid sequences linked by at least one disulfide bond. The present peptide does not include the entire native IGFBP protein but may comprise up to about 250 amino acids of the full length IGFBP. The peptide should be at least about 7 amino acids in length and should comprise certain amino acids needed for IGF binding, as further described herein. Thus, the peptide can be from 7 to 8, 9, 10, 11, 12, 13, 14, 15, 16, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, or 140, 145, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250 and any length in between.

25

It is understood that an "isolated peptide" can include a recombinant peptide. One skilled in the art would recognize that to express correctly folded disulfide-bonded C-terminal fragments of IGFBP as a secreted protein, a 5' segment of the IGFBP cDNA containing the sequence for the signal peptide and the initial N-terminal amino acid of the mature protein should be generated using techniques known in the art, and as shown

in Figure 14. An isolated peptide can also be made using chemical synthesis, isolation techniques, or by cleavage from a larger peptide.

It is also understood that "an isolated peptide" can include certain

5 modifications, including, for example, replacement of the free carboxylic acid at the C-terminal with a C-terminal amide, cyclization of the peptide, introduction of alternative flanking residues, introduction of non-naturally occurring amino acids, or replacement of amino acids with conformational constrained amino acids or non-naturally occurring amino acids. See, e.g., Scarborough et al, 1993. Thus, wherever an amino acid

10 sequence is provided, for example, WHKPRPT (SEQ ID NO:76). Such a sequence includes WHKPRPT-amide, as well as WHKPRPT-COOH. The peptide of SEQ ID NO:84, for example, can be cyclized by introducing an N-terminal cysteine residue and subsequently substituting a penicillamine residue at the C-terminus to result in a constrained ring structure. Thus, "modification," as used throughout, is meant to

15 include peptides or proteins that have variations in amino acid sequence, such as amino acid substitutions, deletions, insertions, or additions or modification of the N- and C-termini (including, for example, addition of an amide group at the C-terminus) or variations in amino acid structure (including, for example, the peptide can be cyclized or conformationally constrained) so long as the peptide or protein retains its desired

20 function. "Modification" can further include the peptide or protein in fragments joined only by disulfide bonds rather than covalently linked, so long as the fragments joined by disulfide bonds retain the desired function as the covalently linked, continuous sequence. For example, the desired function of the binding domain is the ability to bind IGF with high affinity, and modifications thereof should retain that function. In a

25 competitive binding assay with IGF-1, the IC_{50} values (K_D) for the binding domain should be no greater than 100 nM, and preferably between 100 nM and 0.01 nM. Even more preferably the IC_{50} value should be about 1.0 nM. Such variations in the amino acid sequence may arise naturally as allelic variations (e.g., due to genetic polymorphism) or may be produced by human intervention (e.g., by mutagenesis of

cloned DNA sequences), such as induced point, deletion, insertion and substitution mutants. When such variations occur, minor changes in amino acid sequence are generally preferred, such as conservative amino acid replacements, small internal deletions or insertions, and additions or deletions at the ends of the molecules.

- 5 Substitutions may be designed based on, for example, the model of Dayhoff, *et al.*, 1978. These modifications can result in changes in the amino acid sequence, provide silent mutations, modify a restriction site, or provide other specific mutations. One example of a modification includes, for example, a substitution of amino acid residue 281, wherein the arginine residue of the native IGFBP-2 is substituted with a cysteine, 10 which has no effect on the binding activity of the peptide. Furthermore, other substitutions (one amino acid with an aliphatic side chain for another with an aliphatic side chain; one amino acid with an acidic side chain for another with an acidic side chain; etc.) will be obvious to those skilled in the art. For example, a substitution of glutamate for aspartate at position 267 of IGFBP-2 would be an obvious modification 15 to one skilled in the art. Methods of testing the modifications suggested herein are also taught in the Examples of the present application.

- As used herein, a "binding domain" includes the docking-site for IGF on the three dimensional IGFBP structure. The binding domain includes a contact site of 20 IGFBP. By "contact site" is meant the position of covalent insertion. For IGFBP-2 the contact site identified with the photoprobe N^αGly¹(4-azidobenzoyl)IGF-1 (hereinafter "N^αGly¹AB-IGF-1") is glutamine 277. One skilled in the art would recognize that the contact site for IGF without the labeled extension (i.e., without the photoprobe, as described below) could be residues adjacent to or near the glutamine 277. Those 25 skilled in the art would also recognize that the contact site on other IGFBPs would be in the region homologous to glutamine 277 and residues near or adjacent to the homologous site, as shown in Figure 4. Thus, the C terminal regions of the IGFBPs are shown herein to include the IGF binding domains, and one skilled in the art would recognize the homologous regions in the various IGFBPs.

By "about the same binding affinity as the full length IGFBP" is meant an affinity that is within one log unit of the binding affinity of native or recombinant IGFBP, which is about 0.3 nM in a competitive binding assay with IGF. Thus, the binding domain of the present invention comprises the amino acid sequence necessary
5 for high affinity binding. One skilled in the art would recognize that high affinity binding would require a certain three dimensional structure in the binding domain. A schematic depiction of one example of the IGFBP-binding domain on IGF-1 is shown in Figure 1.

10 The IGFBP can be IGFBP-2. More specifically, the present invention provides the isolated IGF binding domain of IGFBP-2 or modifications thereof comprising an amino acid sequence selected from the group consisting of ERGPLEHLYSLHIPNC (SEQ ID NO:84), ERGPLEHLYSLHIPNCD (SEQ ID NO:85), ERGPLEHLYSLHIPNCDK (SEQ ID NO:86), ERGPLEHLYSLHIPNCDKH (SEQ ID
15 NO:87), ERGPLEHLYSLHIPNCDKHG (SEQ ID NO:88), ERGPLEHLYSLHIPNCDKHGL (SEQ ID NO:89), ERGPLEHLYSLHIPNCDKHGLY (SEQ ID NO:90), ERGPLEHLYSLHIPNCDKHGLYN (SEQ ID NO:91), ERGPLEHLYSLHIPNCDKHGLYNL (SEQ ID NO:92),
20 ERGPLEHLYSLHIPNCDKHGLYNLK (SEQ ID NO:93), ERGPLEHLYSLHIPNCDKHGLYNLKQ (SEQ ID NO:94), ERGPLEHLYSLHIPNCDKHGLYNLKQC (SEQ ID NO:95), ERGPLEHLYSLHIPNCDKHGLYNLKQCK (SEQ ID NO:96), ERGPLEHLYSLHIPNCDKHGLYNLKQCKM (SEQ ID NO:97),
25 ERGPLEHLYSLHIPNCDKHGLYNLKQCKMS (SEQ ID NO:98), ERGPLEHLYSLHIPNCDKHGLYNLKQCKMSL (SEQ ID NO:99), ERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLN (SEQ ID NO:100), ERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNG (SEQ ID NO:101), ERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQ (SEQ ID NO:102),

- ERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQR (SEQ ID NO:103),
ERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRG (SEQ ID NO:104),
ERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGE (SEQ ID NO:105),
ERGPLEHLYSLHIPN (SEQ ID NO:106), ERGPLEHLYSLHIP (SEQ ID NO:107),
5 ERGPLEHLYSLHI (SEQ ID NO:108), ERGPLEHLYSLH (SEQ ID NO:109),
ERGPLEHLYSL (SEQ ID NO:110), ERGPLEHLYS (SEQ ID NO:111),
ERGPLEHLYSLHIPNMDKHGLYNLKQCKMSLNGQRGECW (SEQ ID NO:130),
QQELDQVLERISTMRLPDERGPLEHLYSLHIPNC (SEQ ID NO:131),
GPLEHLYSLH (SEQ ID NO:132), IPNCDKHGLY (SEQ ID NO:133),
10 NLKQCKMSLN (SEQ ID NO:134), MSLNGQRGEC (SEQ ID NO:135),
KHGLYNLKQC (SEQ ID NO:136), LYSLHIPNCD (SEQ ID NO:137),
LPDERGPLEH (SEQ ID NO:138), ISTMRLPDER (SEQ ID NO:139), QVLERISTMR
(SEQ ID NO:140), QQELDQVLER (SEQ ID NO:141),
GPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTI
15 RGDPECHLFYNEQR (SEQ ID NO:1),
GPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTI
RGDPECHLFYNEQ (SEQ ID NO:2),
PLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIR
GDPECHLFYNEQR (SEQ ID NO:3),
20 PLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIR
GDPECHLFYNEQ (SEQ ID NO:4),
LEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRG
DPECHLFYNEQR (SEQ ID NO:5),
LEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRG
25 DPECHLFYNEQ (SEQ ID NO:6),
EHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGD
PECHLFYNEQR (SEQ ID NO:7),
EHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGD
PECHLFYNEQ (SEQ ID NO:8),

- HL YSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDP
ECHLFYNEQR (SEQ ID NO:9),
HL YSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDP
ECHLFYNEQ (SEQ ID NO:10),
5 L YSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPE
CHLFYNEQR (SEQ ID NO:11),
L YSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPE
CHLFYNEQ (SEQ ID NO:12),
YSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPEC
10 HLFYNEQR (SEQ ID NO:13),
YSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPEC
HLFYNEQ (SEQ ID NO:14),
SLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECH
LFYNEQR (SEQ ID NO:15),
15 SLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECH
LFYNEQ (SEQ ID NO:16),
LHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHL
FYNEQR (SEQ ID NO:17),
LHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHL
20 FYNEQ (SEQ ID NO:18),
HIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLF
YNEQR (SEQ ID NO:19),
HIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLF
YNEQ (SEQ ID NO:20),
25 IPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFY
NEQR (SEQ ID NO:21),
IPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFY
NEQ (SEQ ID NO:22),

- PNC DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFY
NEQR (SEQ ID NO:23),
PNC DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFY
NEQ (SEQ ID NO:24),
- 5 NCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYN
EQR (SEQ ID NO:25),
NCDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYN
EQ (SEQ ID NO:26),
CDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNE
- 10 QR (SEQ ID NO:27),
CDKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNE
Q (SEQ ID NO:28),
DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNEQ
(SEQ ID NO:29),
- 15 DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNEQ
QEA (SEQ ID NO:30), DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQG
APTIRGDPECHLFYNEQQEA C/R G (SEQ ID NO:31),
DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNEQ
QEA C/R GVH (SEQ ID NO:32),
- 20 DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNEQ
QEA C/R GVHTQ (SEQ ID NO:33), or
DKHGLYNLKQCKMSLNGQRGECWCVPNTGKLIQGAPTIRGDPECHLFYNEQ
QEA C/R GVHTQRMQ (SEQ ID NO:34). Preferably, the isolated IGF binding
domain of IGFBP-2 comprises ERGPLEHLYSLHIPNC (SEQ ID NO:84).

25

The isolated IGF binding domain of IGFBP-2 comprising amino acid sequence
SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88,
SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93,
SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98,

SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, and SEQ ID NO:34 can have at least one intrasequence disulfide bond. The intrasequence disulfide bond can be between the cysteine residues corresponding to residues 249 and 270 of IGFBP-2 or between the cysteine residues corresponding to residues 236 and 247 of IGFBP-2. Preferably, there are two intrasequence disulfide bonds between the cysteine residues corresponding to residues 249 and 270 and between the cysteine residues corresponding to residues 236 and 247 of IGFBP-2. See, for example, the amino acid sequences shown in Figures 6-13 with two disulfide bonds.

20

Another embodiment of the isolated binding domain comprises the amino acid sequence

ELAVFREKVTEQHRQMGKGGKHHLGLEEPKKLRPPPARTPCQQELDQVLERIS
TMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTG

25 KLIQGAPTIRGCPECHLFYNEQQEACGVHTQRMQ (SEQ ID NO:35) with intrasequence disulfide bonds as shown in Figure 7. Another embodiment comprises the amino acid sequence

TPCQQELDQVLERISTMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLN
QGRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQQEACGFHTQRMQ (SEQ

ID NO:36) with intrasequence disulfide bonds as shown in Figure 8.

DKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHL (SEQ ID NO:37) with intrasequence disulfide bonds as shown in Figure 10, or

NLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHL (SEQ ID NO:38)

5 with intrasequence disulfide bonds as shown in Figure 11. Other embodiments include the amino acid sequence

GPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTI
RGDPECHLFYNEQQEACGVHTQR (SEQ ID NO:117),

NLKQCKMSLNGQRGECWCKMSLNGQRGECWCKMSLNGQRGECWCKMSLN

10 GQRGECW (SEQ ID NO:39), or

WCVNPNTGKLIQGAPTIRGDPECWCVNPNTGKLIQGAPTIRGDPECWCVNPNT
GKLIQGAPTIRGDPECWCVNPNTGKLIQGAPTIRGDPECHL (SEQ ID NO:40)

with intrasequence disulfide bonds as shown in Figures 6, 12 and 13, respectively.

15 One skilled in the art would recognize that modified forms, including truncated forms, of the sequences would also bind IGF with high affinity. Thus, a binding domain can comprise or consist of a first sequence, CKMSLNGQRGECWC (SEQ ID NO:41), and a second sequence comprising CHLFYNEQ (SEQ ID NO:42), wherein the sequences are linked by at least one disulfide bond. Furthermore, the C-terminal end of
20 each amino acid sequence could be modified to include a C-terminal amide rather than a free carboxylic acid.

The present invention also provides the isolated IGF binding domains of IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-5, and IGFBP-6. One skilled in the art could
25 identify the regions in IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6 homologous to the binding domain of IGFBP-2, according to Figure 4. Preferably, the IGF binding domain of IGFBP-2 comprises, for example, the amino acid sequence of ERGPLEHLYSLHIPNC (SEQ ID NO:84). The corresponding IGF binding domain of IGFBP-1 comprises GEEISKFYLPNC (SEQ ID NO:112), of IGFBP-3 comprises

PRGVHIPNC (SEQ ID NO:113), of IGFBP-4 comprises HEDLYIIPNC (SEQ ID NO:114), of IGFBP-5 comprises PRAVYLPNC (SEQ ID NO:115), and IGFBP-6 comprises EVYRGAQTLYVPNC (SEQ ID NO:116). Alternatively, when the IGF binding domain of IGFBP-2 comprises, for example, SEQ ID NO: 29, the

5 corresponding IGF binding domain of IGFBP-1 comprises the amino acid sequence of NKNGFYHSRQCETSM DGEAGLCWCVYPWNGKRIPGSPEIRGDPNCQIYFNVQ (SEQ ID NO:43) and the IGF binding domain of IGFBP-3 comprises the amino acid sequence of

10 DKKGFYKKKQCRPSKGRKRGFCWCVDKYGQPLPGYTTKGKEDVHCYSMQSK (SEQ ID NO:44) linked by a disulfide bond. The IGF binding domain of IGFBP-4 comprises the amino acid sequence of

DRNGNFHPKQCHPALDGQRGKCWCVDRTGTVKLPGGLEPKGELDCHQLADS FRE (SEQ ID NO:45). The IGF binding domain of IGFBP-5 comprises

15 DRKGFYKRKQCKPSRGRKRGICWCVDKYGMKLPGMEYVDGDFQCHTFDSSN VE (SEQ ID NO:46). The IGF binding domain of IGFBP-6 comprises

DHRGFYRKRQCRSSQGQRRGPCWCVDRMGKSLPGSPDGNGSSSCPTGSSG (SEQ ID NO:47). As with the IGF binding domain of IGFBP-2, the amino acid sequences comprising the IGF binding domain of the other IGFBP's could include

20 modified forms so long as the binding affinity of the binding domain is about the same as that of the comparable native full length IGFBP. Examples of these binding domain peptides include sequences from the other IGFBP's that are homologous to the sequences of IGFBP-2. As discussed above for IGFBP-2, one skilled in the art would recognize that modified forms of the other IGFBPs would bind IGF with high affinity. For example, one skilled in the art would recognize that the amino acid corresponding

25 to residue number 267 could be serine, aspartate, or glutamate in the various IGFBPs. See Figure 4.

It is understood that the present invention can be based on mammalian IGFBP and IGF, and more specifically, human IGFBP and IGF. The amino acid sequences of these proteins are either known in the art or readily available.

5 The isolated binding domain or modification thereof of the present invention can have a molecular weight of about 14 kDa or less based on MALDI-TOF mass spectrometry. The 14 kDa fragment can contain pieces of the C-terminus of IGFBP-2, disulfide linked to upstream fragments. Under reducing conditions, at least about 3 or 4 peptide sequences can be obtained from the 14 kDa fragment. In an alternative
10 embodiment, the isolated binding domain or modification thereof has a molecular weight of about 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.5, 1 kDa or less, including any amount in between these weights. The present invention provides a polypeptide comprising the IGF binding domain of IGFBP, wherein the polypeptide can range from about 7 to 250 amino acids.

15

 The present invention also provides an IGF antagonist, comprising a peptide of 250 or fewer amino acids, wherein the peptide can displace bound IGFBP from IGF and wherein the peptide reduces binding of IGF to an IGF receptor. Further provided is the IGF antagonist, wherein the antagonist is not CCYAAPLKPAKSC (SEQ ID
20 NO:142). Preferably, the IGF antagonist does not reduce binding of insulin to an insulin receptor. The molecular weight of the antagonist can be 0.5 to 14 kDa. In one embodiment, the IGF antagonist, comprising an isolated peptide comprising an IGF binding domain of an IGFBP or modification or fragment thereof, which reduces binding of IGF to an IGF receptor. "Reduces binding of insulin-like growth factor to an
25 insulin-like growth factor receptor" includes reduction in the range of 5-100% reduction in the binding of IGF to the IGF receptor. The preferred reduction by the IGF antagonist will decrease the biologic activity of IGF. Thus, preferably, the reduction will be 100%, 99%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5%. To decrease the biologic activity

of IGF, the IGF antagonist can bind to IGF and interfere with the availability of IGF for binding to the IGF receptor or can alter the structure of IGF, thereby reducing its ability to bind to the IGF receptor. Furthermore, the IGF antagonist of the present invention can comprise the IGF binding domain of IGFBP or fragments or modifications thereof, as described above and shown in Figures 6-13. Preferably, the IGF antagonist does not reduce binding of insulin to an insulin receptor by greater than about 15 %, and more preferably about 5%. Thus, in the presence of the IGF antagonist, the binding affinity of insulin to the insulin receptor will be about 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% of the normal IC_{50} , which is approximately 1nM. In addition, smaller fragments of the IGF binding domain on IGFBP that act as IGF antagonists can comprise the amino acid sequence of SEQ ID NO:84 or modification thereof. Additionally, the IGF antagonist can comprises other fragments of the binding domain including, for example, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, SEQ ID NO:141, SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO: 3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, or SEQ ID NO:34, FYNEQ (SEQ ID NO:48), PECHLFYNEQ (SEQ ID NO:49), TIRGDPECHLFYNEQ (SEQ ID NO:50), IQGAPTIRGDPECHLFYNEQ (SEQ ID NO:51),

- NTGKLIQGAPTIRGDPECHLFYNEQ (SEQ ID NO:52),
WCVNPNTGKLIQGAPTIRGDPECHLFYNEQ (SEQ ID NO:53),
QRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQ (SEQ ID NO:54),
MSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQ (SEQ ID NO:55),
5 LKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQ (SEQ ID
NO:56),
GLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQ (SEQ
ID NO:57) or modifications thereof. These amino acid sequences are isolated regions
of IGFBP-2. One skilled in the art would recognize that comparable fragments of
10 IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6 would act as IGF antagonists. For
example, the fragments of IGFBP-1, IGFBP-3, IGFBP-4, IGFBP-5, and IGFBP-6 that
are comparable to SEQ ID NO:84 and that would act as IGF antagonists are
GEEISKFYLPNC (SEQ ID NO:112), PRGVHIPNC (SEQ ID NO:113),
HEDLYIIPNC (SEQ ID NO:114), PRAVYLPNC (SEQ ID NO:115), and
15 EVYRGAQTLVPNC (SEQ ID NO:116), respectively. Any of these peptide
fragments that act as antagonists can be modified as described herein.

- In an alternative embodiment, the invention provides an IGF antagonist that is
not a fragment of IGFBP-2, but which can displace IGFBP, bind to IGF, and reduce
20 binding of IGF to an IGF receptor. Preferably, the IGF antagonist does not reduce
binding of insulin to an insulin receptor. Preferably, the IGF antagonist can comprise
as few as three amino acids. Even more, preferably, the IGF antagonist can comprise a
tripeptide, tetrapeptide, pentapeptide, hexapeptide, or heptapeptide. Alternatively, the
antagonist can comprise 9, 10, 11, 12, 13, 14, 15, 16, amino acids or any number of
25 residues less than 250. Preferably, the IGF antagonist is selected during screening of a
peptide library using the Phage Display technique. Even more preferably, the IGF
antagonist comprises a peptide that complies with the consensus sequence WHKPRPR
(SEQ ID NO:79). For example, IGF antagonists that comply with this consensus
sequence and that displace bound IGFBP-2 from IGF-1 during screening of a

heptapeptide library using the Phage Display technique are as follows: FHKHRMP (SEQ ID NO:58), FHKHRTL (SEQ ID NO:59), FHKHRVS (SEQ ID NO:60), FHKPPRL (SEQ ID NO:61), FHKRYPP (SEQ ID NO:62), FHKWPRV (SEQ ID NO:63), IHKHKLR (SEQ ID NO:64), LHKYTKS (SEQ ID NO:65), WHGSWKK (SEQ ID NO:66), WHKHPRA (SEQ ID NO:67), WHKHQRL (SEQ ID NO:68), WHKHTRV (SEQ ID NO:69), WHKHYPYR (SEQ ID NO:70), WHKKPIP (SEQ ID NO:71), WHKKTPP (SEQ ID NO:72), WHKPFRF (SEQ ID NO:73), WHKPRL (SEQ ID NO:74), WHKPRLY (SEQ ID NO:75), WHKPRPT (SEQ ID NO:76), WHKPWIR (SEQ ID NO:77), WHKWPQR (SEQ ID NO:78), and WHKPRPR (SEQ ID NO:79).
10 Preferably, the antagonist comprises SEQ ID NO:76 or SEQ ID NO:79. The antagonists can be modified as described herein.

The present invention also provides a fragment of IGF or modification thereof, wherein the fragment or modification thereof binds to a binding domain of IGFBP.
15 More specifically, the fragment of IGF can be a fragment of an IGF selected from the group consisting of IGF-1 and IGF-2. The IGFBP to which the fragment or modification thereof binds can be selected from the group consisting of IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, and IGFBP-6. Preferably, the fragment of IGF reduces binding of IGF to IGFBP by at least 5%. Thus, the fragment can reduce
20 binding of IGF to IGFBP in the range of 5-100%. Preferably, the reduction will be 100%, 99%, 98%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5%. The fragment of IGF in the present invention can comprise the amino acid sequence of GPETLCG (SEQ ID NO:80) or AYRPSETLCG (SEQ ID NO:81), which are the N-termini of IGF-1 and IGF-2,
25 respectively. The IGF fragment of the claimed invention can further comprise the amino acid sequence of SEQ ID NO:80 or SEQ ID NO:81 linked to the amino acid sequence FRS (SEQ ID NO:82) by a disulfide bond. The amino acid sequence of SEQ ID NO:33 represents residues 49-51 in IGF-1 and IGF-2. A cysteine residue, which is essential for proper folding of IGF, precedes SEQ ID NO:82 in native IGF. One skilled

in the art could identify the homologous regions in IGF-1 and IGF-2, as shown in Figure 2, and identify other fragments of IGF-2 comparable to fragments of IGF-1 (e.g., amino acid residues 1-7 of IGF-1 as represented by SEQ ID NO:80 would correspond to amino acid sequence PSETLCG (SEQ ID NO:83), for example). One skilled in the art would know that the IGF fragment can be modified as discussed above for IGFBP, so long as the fragments retain the ability to bind to IGFBP. For example, the glutamate corresponding to residue three of IGF-1 could be substituted with an aspartate, another amino acid with an acidic side chain. One skilled in the art would recognize similar substitutions and modifications as described above.

10

The present invention also provides a method of screening for IGFBP antagonists comprising screening a peptide library using a Phage Display technique and identifying a peptide that displaces bound IGF from IGFBP or a fragment thereof, wherein the peptide that displaces bound IGF from IGFBP is an IGFBP antagonist. The fragment of IGFBP can comprise the peptide of SEQ ID NO:84. Further provided by the invention is an IGFBP antagonist comprising a peptide identified by the present method or comprising a peptide that is a fragment of IGF.

The present invention also provides a method of screening for IGF antagonists comprising screening a peptide library using a Phage Display technique and identifying a peptide that displaces bound IGFBP from IGF or a fragment thereof, wherein the peptide that displaces bound IGFBP from IGF or a fragment thereof is an IGF antagonist. The biotinylated IGF of the present invention can be used in the method of screening for IGF antagonists.

25

The present invention further provides a protein complex, comprising a peptide containing the IGF binding domain of IGFBP of the invention and an IGF fragment of the invention. The present invention also provides a protein complex, comprising a peptide containing the IGF binding domain of IGFBP and a photoaffinity derivative of

an IGF. Specifically the photoaffinity derivative can comprise a photoreactive glycine residue within an IGFBP binding domain of IGF. More specifically, the photoreactive glycine residue can be an N-terminal glycine residue. Even more specifically, the N-terminal derivative can be N^{αGly} AB-IGF-1.

5

The present invention provides a method of treating a subject with cancer, comprising administering to the subject an IGFBP binding domain peptide, wherein the peptide is preferably an IGF antagonist. The cancer can comprise a tumor that expresses an IGF receptor, more specifically, the cancer can include breast cancer or prostate cancer. The efficacy of the treatment can be evidenced by a reduction in clinical manifestations or symptoms, including, for example, the size of a tumor or reductions in the amount of IGF available for binding to an IGF receptor of the tumor. Examples of these protocols are well known in the art. For example, the fragment can be administered to subjects having an IGF-dependent tumor, and tumor size could be monitored using imaging techniques, such as MRI, mammography, or ultrasound depending on the type of tumor. Imaging could be performed, for example, twice monthly. Serum levels of IGF and IGFBP could also be measured from serum samples from the subject at regular intervals.

10
15

For example, levels of plasma IGF-1 and IGF-2 in treated subjects can be monitored with radioimmunoassay, using an antibody specific for IGF and preferably for the IGFBP binding domain on IGF. Plasma IGF levels could be measured with and without acid dissociation of IGFs and IGFBPs in order to assess the levels of bound and unbound IGF. Thus, by comparing the IGF levels with and without acid dissociation, the amount of unbound IGF can be determined. Normal serum levels of IGF-1 and IGF-2 after acid dissociation typically range from about 90 to 320 and 288-740 μg/L, respectively. Plasma levels of the IGF antagonist can be assessed similarly using a high affinity monoclonal antibody specific for the IGF antagonist.

20
25

"Subject" as used herein can refer to any vertebrate but preferably refers to a mammal, and, more preferably, to a human.

Also, the present invention provides a method of preventing cancer in a subject,
5 comprising administering to the subject a peptide of the invention that is an IGF
antagonist. As used herein, "preventing" means avoiding, reducing, or delaying a
process related to a disease or injury. More specifically, the cancer can comprise a
tumor that expresses an IGF receptor, including breast cancer or prostate cancer. Even
more specifically, the IGF receptor expressed by the tumor can be an IGF-1 receptor.
10 Subjects for whom the preventive measures are appropriate include those with one or
more known risk factors for cancer.

The present invention further provides a method of treating a subject with a
diabetic complication exacerbated by IGF, comprising administering to the an IGF
15 antagonist. Specifically, the diabetic complication can include diabetic retinopathy or
diabetic nephropathy. Also provided are methods of preventing diabetic complications
exacerbated by IGF, comprising administering to the subject an IGF antagonist. The
efficacy of the treatment can be evidenced by a reduction in clinical manifestations or
symptoms, including, for example, improved renal clearance, improved vision, or a
20 reduction in the amount of IGF available for binding to the IGF receptor.

Further provided is a method of treating a subject with an ischemic injury,
comprising administering to the subject the IGF fragment or IGFBP antagonist of the
invention, thereby reducing the ischemic injury in the subject. The ischemic injury can
25 be reduced indirectly by the IGF fragment or IGFBP antagonist binding to the IGFBPs,
thereby increasing the IGF available for binding to the IGF receptor. Ischemic injuries
can include strokes, myocardial ischemia, and ischemic injury to the kidneys. The IGF
fragment or IGFBP antagonist of the claimed invention can also be used in methods of
treating subjects with other diseases and injuries, wherein higher circulating levels of

IGF are desired. For example, a method of treating a subject with diabetes is provided, comprising administering to the subject the IGF fragment or IGFBP antagonist, thereby reducing blood glucose levels in the subject. Other diseases or injuries that can also be treated with the IGF fragment or IGFBP antagonist of the claimed invention include

5 neurologic diseases and injuries, including, for example, Parkinson's disease, amyotrophic lateral sclerosis, head trauma, multiple sclerosis and other neurologic disorders. IGF receptor activation can increase neurite sprouting and thereby provide clinical benefits. Also included are diseases in which building bone and connective tissue is desired, including, for example, osteoporosis and arthritis. Similarly,

10 administration of the IGF fragment or IGFBP antagonist of the claimed invention could be used to prevent or reduce the effects of ischemic injury or diseases characterized by reduced IGF activation of the IGF receptor.

The peptides of the present invention can be administered orally, parenterally,

15 intraventricularly, topically, transdermally, transdermally, extracorporeally, topically or the like, although oral or topical administration is typically preferred. Parenteral administration of the peptides of the present invention, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid

20 prior to injection, or as emulsions. As used herein, "parenteral administration" includes intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous and intratracheal routes. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. The

25 fragments can also be administered using polymer based delivery systems, including, for example, microencapsulation as described in Langer. The peptides of the present invention can be administered using gene therapy methods of delivery. See, e.g., U.S. Patent No. 5,399,346, which is incorporated by reference herein. Using a gene therapy method of delivery, primary cells transfected with the gene for the peptides of the

present invention can additionally be transfected with tissue specific promoters or tumor specific promoters to target specific organs or tissue, including, for example, kidney or breast. An example of a tissue specific promoter for breast is MMTV.

5 The exact amount of such peptides required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the disease that is being treated, the particular compound used, its mode of administration, and the like. A preferred range for doses of the peptides of the present invention is 1.0-1,000 mg/kg of body weight once per day. More preferably, the
10 dosage range for the IGF antagonist of the present invention is 0.1 to 10 mg/kg of body weight when administered subcutaneously or intramuscularly. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See *Remington's Pharmaceutical Sciences* (Martin, E.W., ed., latest edition), Mack Publishing Co., Easton, PA.

15

 Depending on the intended mode of administration, the peptides of the present invention can be in pharmaceutical compositions in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, suppositories, pills, capsules, powders, liquids, suspensions, lotions, creams, gels, or the like, preferably in unit
20 dosage form suitable for single administration of a precise dosage. The compositions will include, as noted above, an effective amount of the selected antagonist or fragment in combination with a pharmaceutically acceptable carrier and, in addition, may include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise
25 undesirable, i.e., the material may be administered to an individual along with the selected antagonist or fragment without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

For solid compositions, conventional nontoxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talc, cellulose, glucose, sucrose, magnesium carbonate, and the like. Liquid pharmaceutically administrable compositions can, for example, be prepared by
5 dissolving, dispersing, etc. an active compound as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH
10 buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see *Remington's Pharmaceutical Sciences*, E.W. Martin, (ed.), Mack Publishing Co., Easton, Pennsylvania, U.S.A.

15

For oral administration, fine powders or granules may contain diluting, dispersing, and/or surface active agents, and may be presented in water or in a syrup, in capsules or sachets in the dry state, or in a nonaqueous solution or suspension wherein suspending agents may be included, in tablets wherein binders and lubricants may be
20 included, or in a suspension in water or a syrup. Where desirable or necessary, flavoring, preserving, suspending, thickening, or emulsifying agents may be included. Tablets and granules are preferred oral administration forms, and these may be coated.

Parenteral administration, if used, is generally characterized by injection.
25 Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system, such that a constant level of dosage is maintained.

The present invention provides a nucleic acid that encodes a peptide comprising an IGF binding domain on an IGFBP or modification thereof, which binds IGF with at least about the same binding affinity as the full length IGFBP. The present invention

5 also provides a means for expressing a recombinant peptide comprising an IGF binding domain on an IGFBP or modification thereof, which binds IGF with at least about the same binding affinity as the full length IGFBP. Specifically, a vector for the expression of the peptide, comprising the nucleic acid of the present invention, is provided. The nucleic acid can be in any vector of choice, such as a plasmid or a viral vector, and the

10 method of transfer into the cell can be chosen accordingly. More specifically, the vector can be a plasmid. Even more specifically, the vector can comprise a promoter functionally linked to one of the nucleic acids of the present invention. "Vector" means any carrier containing foreign DNA. "Vectors" include but are not limited to plasmids, viral nucleic acids, viruses, phage nucleic acids, phages, cosmids, and artificial

15 chromosomes. As known in the art, nucleic acids can be modified for particular expression, such as by using a particular cell- or tissue-specific promoter, by using a promoter that can be readily induced, or by selecting a particularly strong promoter, if desired. One skilled in the art would recognize that to achieve expression of a correctly folded/disulfide-bonded binding domain from the C-terminal of a protein, the sequence

20 encoding the signal peptide and the initial N-terminal amino acids of the mature protein would also need to be included. The sequences encoding the initial N-terminal amino acids necessary to achieve expression of a correctly folded/disulfide-bonded binding domain would preferably include the sequences for the initial 10 amino acids of the N-terminal region of the mature IGFBP; more preferably, the sequences for 9, 8, 7, 6, 5, 4,

25 3, 2, or 1 amino acids of the N-terminal region of mature IGFBP would be included.

Also provided is a cell containing an exogenous nucleic acid comprising one of the nucleic acids of the present invention. A method of expressing the recombinant protein produced by the transformed cells comprising optimizing active IGF binding by

the recombinant protein is also provided. More specifically, the cell can be an *Escherichia coli*, yeast cell, or CHO cell.

The invention also provides an isolated IGF-1, wherein one residue of the IGF-1 is biotinylated. and wherein the biotinylated IGF-1 has a high affinity for IGFBP-2. Preferably, the affinity of the biotinylated IGF-1 for IGFBP-2 is comparable to the affinity of unmodified IGF-1 for IGFBP-2, and more preferably in the range of about 300 to 50 pM (K_D) in a competition binding assay. Preferably the biotinylated IGF-1 is selected from the group consisting of N^αGly1-, N^εLys65-, and N^εLys27- monobiotinylated IGF-1. The monobiotinylated IGF-1 can be homogeneous or various monobiotinylated isoforms can be combined; however, the biotinylated IGF-1 is relatively free of tribiotinylated and tetrabiotinylated IGF-1. By relatively free of tribiotinylated and tetrabiotinylated IGF-1 is meant that more than 80-100% of the biotinylated IGF-1 is monobiotinylated or dibiotinylated and that no more than about 20% of the biotinylated IGF-1 is tribiotinylated or tetrabiotinylated.

The following examples are intended to illustrate, but not limit, the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may be alternatively employed.

As used in the specification and in the claims, "a" can mean one or more, depending upon the context in which it is used.

EXAMPLES

EXAMPLE 1

Photoaffinity Derivative of IGF

The N-terminal glycine α -NH₂-group of IGF-1 was derivatized with the photoaffinity reagent, N-hydroxysuccinimido-4-azidobenzoate (HSAB) to yield an IGF-1 photoprobe capable of selectively labeling the IGF-1 binding domain on the IGFBPs (*See* Galaray, 1974). The advantage of this approach is that the photoreactive group is present within the region shown to be essential for IGF-1 binding to the IGFBPs (*See* Szabo, 1988). In this report we describe the synthesis and characterization of N^[α Gly]AB-IGF-1.

Materials Human recombinant IGF-1 was generously provided by Genentech, Inc. (South San Francisco, CA). HSAB was synthesized from para-aminobenzoic acid, sodium azide (Sigma Chemicals, Inc. St. Louis, MO) and dicyclohexyl carbodiimide (Pierce Chemical Co, Rockford, IL) and purified according to the method described by Galaray et al. (1974). The cDNA encoding hIGFBP-2 was obtained from Joerg Landwehr, Roche Biotech, Basel, SZ. Affi-gel agarose was from BioRad. HPLC columns were from Vydac Instruments (Hesperia, CA). All other materials were of reagent grade.

Synthesis and Purification of N^[α Gly]AB-IGF-1. Recombinant human IGF-1 (Genentech; 1 mg; 130 nmol) was reacted with a 10- fold molar excess of HSAB (0.34 mg; 1.3 μ mol) for 1 h at 23 °C. Unreacted ester was quenched by the addition of 30 μ l of ethanolamine for 30 min and the entire reaction mixture was lyophilized. It should be noted that all derivatizations and handling of photoprobes were carried out under subdued lighting or under a red safety light. The lyophilized reaction mixture was dissolved in 0.5 M acetic acid and 10 μ l of trifluoroacetic acid (TFA) and chromatographed on a C18 column equilibrated in 0.1% TFA/24% acetonitrile at a flow rate of 1 ml/min. After 20 min a linear gradient from 24-60% acetonitrile was developed over 60 min to elute IGF-1 and the reaction products.

In addition to unreacted IGF-1 (peak 1), 3 major products are obtained. Analysis of these peaks by acidic polyacrylamide gel electrophoresis in 8 M urea has revealed that all three derivatized IGF-1s are monosubstituted, thus exhibiting the loss of 1 net positive charge. Peak 2 represents the elution position of N^εLys²⁷(azidobenzoyl)IGF-1 (See Yip, 1993). Peaks 3 and 4 have been identified as N^εLys⁶⁸(azidobenzoyl)IGF-1 and N^αGly¹ AB-IGF-1, respectively based on amino acid sequencing analysis.

Peak 4 was subsequently collected from two separate reactions, pooled, dried in a Speed Vac (Savant Instruments, Farmingdale, NY) and further purified on a C18 column equilibrated in 50 mM triethanolamine-phosphate, pH 3.0 and eluted with a gradient of 27.5-38% acetonitrile. The peak was collected and its identity as N^αGly¹ AB-IGF-1 was verified.

Pepsin Digestion of N^αGly¹ AB-IGF-1 IGF-1 and N^αGly¹ AB-IGF-1 were digested with pepsin (at an enzyme:substrate ratio of 1:20) in 0.01M HCl for 5 h at room temperature (See Huynh, 1996). The sample was then injected onto a C18 column equilibrated in 0.1% TFA and the fragments were eluted using a linear gradient of 0 to 60% acetonitrile over 90 min. The pepsin cleavage sites and the AE fragment are shown in Figure 3. The AE fragment for N^αGly¹ AB-IGF-1, which exhibited a significant shift in retention time compared to the underivatized IGF-1 was analyzed by MALDI. Further analysis of this fragment on a Finnigan-Mat LCQ ion trap MS confirmed the predicted sequence. These results clearly indicate that the azidobenzoyl derivative of IGF-1 eluting as peak 4 in the HPLC column profile is N^αGly¹ AB-IGF-1 and that it is photoactivatable.

EXAMPLE 2

Identification and Characterization of the IGF binding domain on rhIGFBP-2

Preparation and Purification of rhIGFBP-2 A Chinese hamster ovary (CHO) cell expression system was used for its convenience and high yield of correctly folded,

functional protein (approximately 0.5 to 1 liter of conditioned medium per week with a concentration of rhIGFBP-2 of 3 mg/L as the final yield of purified protein).

Dihydrofolate reductase (DHFR) minus CHO cells were cotransfected with cDNAs encoding DHFR and hIGFBP-2 (Joerg Landwehr, Basel, Switzerland). Stable
5 transfectants were selected in nucleoside-free medium. The transfected genes were subsequently amplified by exposing the cells to increasing doses of methotrexate. The highest rhIGFBP-2 producing clone, based on Coomassie staining of equal aliquots of medium was expanded. Cells are grown in roller bottles and the medium is collected from serum-starved cells weekly. Conditioned medium (CM) was dialyzed against
10 0.1% acetic acid and lyophilized. The lyophilized proteins were subsequently purified by sequential IGF-1-agarose affinity chromatography and reversed phase HPLC.

rhIGFBP-2 elution profile on C4 reverse phase column Lyophilized

conditioned medium was dissolved in 50 mM HEPES, pH 7.4 containing 0.15 M NaCl
15 (column buffer) and added to a slurry (5 ml) of IGF-1-agarose. This mixture was gently rocked for 24 h at 4°C, packed into a column and washed with 20 ml of column buffer followed by elution with 1.0 M acetic acid (10 ml). The eluate was dried *in vacuo* in a Speed Vac concentrator, dissolved in 0.1% trifluoroacetic acid (TFA) and injected onto a C4 column. Fractions 24 and 27 from the C4 column were resolved on a 10%
20 acrylamide SDS gel under non-reducing conditions and transferred to nitrocellulose. Proteins were visualized by immunoblotting with an anti-IGFBP-2 antibody (Upstate Biotechnology, Inc., Lake Placid), peroxidase-labeled secondary (Chemicon, Temecula, CA) and Enhanced Chemiluminescence (ECL - Amersham, Arlington Heights, IL). The identity of the rhIGFBP-2 generated by the CHO cells was verified by MALDI-
25 TOF MS. These data corroborate with the amino acid sequence/cDNA sequence of the plasmid used to transfect the cells. The concentration of our stock vials of rhIGFBP-2 are determined on the basis of amino acid analysis, which was then correlated with its extinction coefficient, using an absorbance value of 0.713 for a 1 mg/ml solution of rhIGFBP-2 in distilled water.

Elution profile of rhIGFBP-2 following extended incubation For this preparation, conditioned medium was concentrated in an Amicon concentrator and allowed to stand at 4°C for 48 hours before purification. This resulted in increased formation of a fragment having a molecular mass of 15.8 kDa by MALDI-TOF-MS.

- 5 The 15.8 kDa fragment was only detected by immunoblot in gels run under non-reducing conditions with a commercial (UBI) polyclonal antiserum (anti-BP-2). This suggests the fragment is composed of multiple polypeptides linked by disulfide bonds. The fragment was not reactive with a site-specific anti-peptide antibody (anti-peptide) raised against residues 119-128, suggesting that the fragment lacks that epitope.

10

MALDI-TOF-MS profile of rhIGFBP-2 An aliquot of purified rhIGFBP-2 was analyzed by MALDI-TOF-MS to confirm its molecular mass. The spectrum indicates a highly purified protein with an apparent mass of 31,454 Da. The predicted mass is 31,323 Da.

15

MALDI-TOF-MS profile of the rhIGFBP-2 fragment An aliquot of purified fragment was analyzed by MALDI-TOF-MS. The spectrum indicates the presence of a singly charged component of 15,820 Da. The other major peak identified at 7,927 m/z represents the double-charged ion.

20

- Inhibition of cell growth by rhIGFBP-2 MCF-7 human mammary carcinoma cells were used in a proliferation-based bioassay to demonstrate the efficacy of rhIGFBP-2 in blocking both exogenously added IGF-1 and endogenous IGF-2 action. Cells grown in 24- well plates in phenol red free RPMI and 10% fetal bovine serum
- 25 were starved of serum for 24 h, washed with PBS and stimulated as indicated. Purified rhIGFBP-2 was added to the indicated wells at 20 or 100 nM final concentration. The wash and treatments were repeated at 48 h and the cells were harvested and counted at 96 h. Shown are average cell numbers as a percent of control (unstimulated cells in the absence of rhIGFBP-2) for triplicate wells. rhIGFBP-2 inhibition of an IGF-2:IGF-1R

autocrine loop significantly reduced the basal growth of these cells in the absence of exogenously added IGF-1.

IGFBP-2 binding assay Soluble IGFBP-2 binding assays were carried out using
5 polyethylene glycol precipitation and centrifugation (*See* Bourner, 1992). Briefly, one
nanogram of rhIGFBP-2 is combined with various concentrations of IGF-1 ranging
from 50 pM to 10 nM in binding assay buffer (100 mM HEPES pH 7.4, 44 mM
NaHCO₃, 0.01% BSA, 0.01% Triton X-100, 0.02 % NaN₃) followed by addition of 10
nCi IGF-1 (Amersham Radiochemicals, Clearbrook, IL). After a 4 hr incubation at
10 room temperature, 250 µl 0.5% bovine gamma globulin is added followed by 500 µl
25% polyethylene glycol (avg. MW 8000; Sigma Chemicals, Inc. St. Louis, MO). The
samples are incubated for 10 min at room temperature and centrifuged 3 min at 13
krpm. The pellets were washed with 1 ml 6.25% PEG and counted in a gamma counter
for total counts bound. Counts bound in the presence of 1 µM IGF-1 are subtracted to
15 obtain specific binding.

In soluble competition binding assays, the N^αGly¹AB-IGF-1 displayed an
identical affinity to IGF-1 for binding to rhIGFBP-2. Both were found to have a K_D of
300 pM.

20

Photoaffinity labeling of rhIGFBP-2 Equimolar quantities (7 nmol) of
N^αGly¹AB-IGF-1 and rhIGFBP-2 were allowed to attain equilibrium binding by co-
incubation for 4h at 23°C. The sample was then placed in ice water and irradiated for
120 sec with a uv lamp (300 nm) at a distance of 2 cm (320 nm, 8 watts; Fotodyne hand
25 lamp, Fotodyne Corp.). The sample was boiled in SDS sample buffer and resolved on a
10% acrylamide SDS gel under reducing conditions. The proteins were transferred to
nitrocellulose and probed with monoclonal anti-IGF-1 antibodies. The blot was then
stripped at 60°C in buffer containing SDS and β-mercaptoethanol, washed and
reprobed with polyclonal anti-IGFBP-2 antibodies (both from UBI, Lake Placid).

An aliquot of the sample was then subjected to immunoblot analysis. In addition to rhIGFBP-2 migrating in its normal position of about 32 kDa, a second component migrating at about 40 kDa was observed. The 40 kDa complex also stained with the anti-IGF-1 antibodies, demonstrating that the photoprobe, N^{Gly1} AB-IGF-1, is
5 functional both in terms of its high affinity binding interaction with rhIGFBP-2 and its efficient photoincorporation. In the latter case we estimate that approximately 40% of the bound probe was incorporated into the binding protein.

The photolabeling efficiency of the Gly-1 derivatized IGF-1 photoprobe was
10 compared to the Lys-27 derivatized IGF-1 photoprobe. Equal amounts of rhIGFBP-2 (20 μ g) were incubated with N^{Gly1} AB-IGF-1 or N^{Lys27} AB-IGF-1 for 1 h at 23 °C in the absence or presence of excess IGF-1. The samples were then placed on ice and photolyzed with a 320 nm UV lamp (8 watts) for 120 min. The samples were then resolved on a 10% acrylamide SDS gel under reducing conditions. The gel was stained
15 with copper and scanned with transmitted light. The N^{Gly1} AB-IGF-1 photoprobe consistently demonstrated approximately double the labeling efficiency over the N^{Lys27} AB-IGF-1 probe, consistent with the presumed interactions of the N-terminal of IGF-1 with the binding proteins.

20 The photoincorporation site in rhIGFBP-2 of the N^{Gly1} AB-IGF-1 was identified. One mg of rhIGFBP-2 was photolabeled with 200 μ g of N^{Gly1} AB-IGF-1. Following reduction and alkylation, the IGF-1-IGFBP-2 complex formed was isolated by SDS-PAGE. The complex was identified by copper staining of the gel and the appropriate region was excised. Protein complexes were eluted from the gel by trypsin
25 digestion overnight at 37 °C.

Using MALDI-TOF-MS of IGF-1, rhIGFBP-2 and the IGF-1-IGFBP-2 complex, a loss of the peak with a mass of 2770, corresponding to residues 266-287 of IGFBP-2 and 1-21 of IGF-1 was observed. Instead, a new component with a mass of

5,301 was detected, consisting of the N-terminal tryptic fragment of the photoprobe covalently attached to C-terminal tryptic fragment of IGFBP-2.

Comparison of IGF-1 binding affinities between rhIGFBP-2 and its 15.8 kDa

5 fragment Aliquots (1 ng) of rhIGFBP-2 or the 15.8 kDa fragment were analyzed in a soluble competition binding assays described herein. These exciting preliminary results indicate that the 15.8 kDa fragment retains high affinity binding characteristics of the intact binding protein. These findings underscore the potential for success of our goal of generating low molecular weight peptide-based antagonists of the IGFs. Structural
10 analysis of this fragment will complement our photoaffinity labeling studies on rhIGFBP-2. We are presently evaluating several ways of increasing the production of the 15.8 kDa fragment by the CHO cell cultures. In the process we will determine if additional smaller, functional fragments are generated.

15 HPLC profile of reduced and alkylated rhIGFBP-2 fragment Approximately 50 µg of the 15.8 kDa fragment of rhIGFBP-2 was reduced and alkylated with TCEP and 4- vinylpyridine. Three broad peaks were identified, suggesting that this fragment may be comprised of a small number of relatively large peptides (e.g., three major peptides/domains).

20

Analysis of the tryptic fragments present in the 15.8 kDa fragment An aliquot of the fragment was digested with trypsin. The reaction was stopped with TFA and the tryptic peptides generated were analyzed by MALDI-TOF- MS. The contiguous peptide sequences detected by MALDI are shown in Figure 5. Consistent with our immunoblot
25 data, a fragment containing the epitope was not found. Importantly, as predicted from our photoaffinity labeling studies, the C-terminal region of IGFBP-2 is present in the 15.8 kDa IGFBP-2 fragment. The peptide sequence analysis is shown in Figure 5.

The identified IGF binding domain of IGFBP-2 is shown in Figure 7. The peptide sequences corresponding to residues 226 through 289 of IGFBP-2 constitute the binding domain. This sequence contains disulfide bonds between residue 249 and 270 and between residues 236 and 247. The glutamine residue 277 was the site of attachment for the IGF-1 photoprobe. Residue 281 can be either C or R, without changing the biologic activity of the IGFBP-2. The contact site for the N-terminal Gly residue of the probe is with the sequence comprising residues 266 through 287, whereas the binding domain, necessary for high affinity binding, includes additional upstream and downstream residues as shown. The residues proximal to Q-277 are required for IGF docking to IGFBP-2.

EXAMPLE 3

Using a modification of the method of Gansler et al. (*Am. J. Pathol.* 135: 961-966), the effectiveness of treatment of an IGF-dependent tumor can be assessed using an IGFBP fragment. Adult male athymic mice can be subcutaneously heterotransplanted with either MCF-7 human breast cancer cells or human Wilms tumor cells. After one week, animals with visible nodules can be treated with either the IGFBP fragment or a scrambled peptide as a control. The diameters of the nodules can be measured every 2-3 days for 8 weeks, and serum levels of IGF-1 and IGFBPs quantified. After 8 weeks, the animals can be sacrificed and the tumors examined histologically. The effectiveness of the treatment can be determined based on the ability of the fragment to reduce or prevent the growth of the tumor as compared to the control peptide.

EXAMPLE 4

To express correctly folded/disulfide-bonded C terminal fragments of IGFBP-2 as a secreted protein, a 5' segment of the IGFBP-2 -DNA containing the sequence for the signal peptide and the initial two N-terminal amino acids of the mature protein was generated. Using standard techniques known in the art (*Molecular Cloning*, eds.

Sambrook, Fritsch, and Maniatis, 1989), pCMV-IGFBP2 was digested with *BsgI*, blunt ended and digested with *EcoRI*. The appropriate fragment (BP2N) was gel purified and ligated into pBluescript-SK (Stratagene) and digested with *SmaI* and *EcoRI*.

To generate the 3' segment, PCR primers were synthesized flanking the C-terminal region of interest (i.e., residues 228-289 and 187-289 containing 4 and 6 cysteine residues, respectively). The 5' primer included sequences for a *BamH I* restriction site and for the FLAG epitope, immediately upstream of the native IGFBP-2 sequence. The 3' primer was based on the pCMV backbone sequence downstream of the *XbaI* site 3' to the C-terminus of IGFBP-2. The PCR product was gel purified, digested with *BamH I* and *XbaI* and ligated into SK-BP2N digested with the same enzymes. The new construct was excised intact with *EcoRI*, inserted into pCMV and expressed in CHO cells as described for intact IGFBP-2. The FLAG-BP2 C-terminal proteins were purified over an anti-FLAG affinity column, digested with enterokinase to remove the FLAG and N-terminal components and HPLC purified.

15

EXAMPLE 5

The present example describes biotinylation of IGF-1 and its subsequent purification into isoforms with differing biological activities. Human recombinant IGF-1 (Genentech, Inc., South San Francisco, CA) was reacted with a 2.5-fold molar excess of NHS-biotin (Pierce Chemical Co., Rockford, IL) for 10 min at 23°C in 0.1M NaP, pH 7.4. The reaction was terminated by addition of a 10-fold molar excess of Tris over NHS and the mixture was purified by reverse phase HPLC on a C18 column (300 Å pore size; Vydac Corp., Hesperia, CA) equilibrated in 50 mM triethanolamine-phosphate, pH 3.0, and eluted with a gradient of 27.5-38% acetonitrile. Several peaks were observed, which were later identified to be mono-, di-, tri- and tetra-biotinylated isoforms of biotinylated IGF. Each peak was pooled separately and dried in vacuo on a SpeedVac apparatus (Savant Instrument Co., Garden City, NY.). The peaks were rechromatographed on a C18 column equilibrated in 24% acetonitrile in 0.1% TFA, and eluted with a linear gradient of 24%-48% acetonitrile over 40 min. Purified peaks were

dried and stored at -20°C. Mono-, di-, tri- and tetra-biotinylated isoforms of biotinylated IGF were determined using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), described below, and the three mono-biotinylated isoforms were subjected to trypsin digestion in order to determine
5 precise placement of the biotin. Trypsin digestion was done using protocols standard in the field, and trypsinized samples were resolved on a C18 column equilibrated in 0.1% TFA and eluted with a linear gradient of 0-60% acetonitrile in 0.1% TFA over 100 min, so that each peak could be collected separately.

10 Purified isoforms of biotinylated IGF-1, and trypsinized derivatives of mono-biotinylated IGF1, were analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Dried aliquots of each peak were dissolved in 70% acetonitrile/0.1% TFA and mixed 2:1 with matrix (α -cyano-4-hydroxycinnamic acid) in 70% acetonitrile/0.1% TFA. This mixture was spotted on the
15 sample plate and analyzed on a PerSeptive Biosystems (Framingham, MA) Voyager-DE MALDI-TOF mass spectrometer equipped with a 337 nm laser. A delayed extraction source was operated in linear mode (1.2 m ion flight path, 20 kV accelerating voltage), yielding an instrumental resolution of approximately 700 (full width at half maximum) at m/z 1297.5. One mass spectrum was based on 256 average mass scans. External
20 mass calibration was routinely performed using angiotensin I (MW 1297.5) and bovine insulin (MW 5734.54) as standards. Mass Accuracy was ± 2 Da.

MALDI-TOF-MS analysis of the three mono-biotinylated isoforms of IGF-1 revealed them to be biotinylated at Lys 27, Lys65, and Gly1. The isoform biotinylated
25 at Lys65/68 was found to bind IGFBP-2 with as great an affinity as un-biotinylated IGF, while the tetra-biotinylated isoform had a significantly reduced binding affinity. Thus, purification and use of K^{65/68}-Biotin-IGF-1 in binding assays with IGFBP-2 is safer than use of ¹²⁵I-IGF and binds with affinity equivalent to IGF and ¹²⁵I-IGF, a

surprising improvement over the mixed solutions of biotinylated isoforms described in earlier studies.

EXAMPLE 6

5 Biotinylated IGFs were used to screen a library of randomly generated peptides for peptides capable of competing with IGFBP-2 for binding to IGF. The Phage Display technique, using the Ph.D.-7 Phage Display Kit™ obtained from New England Biolabs, Inc., was used for this screening. A peptide library was obtained consisting of randomly generated peptides inserted into the pIII coat protein of the phage M13. This
10 library was combined with a 250 ng/mL solution of IGF that has been biotinylated at Lys65 (K⁶⁵-Biotin-IGF-1). All phage containing peptides capable of binding IGF-1, including those capable of binding IGF-1 at a site where they can effectively compete with IGFBP-2 for binding, bound the free IGF-1, forming IGF-1-M13 complexes. The IGF-1-M13 complexes, and unbound M13, were placed on a Avidin-coated tissue
15 culture plate, to which only the IGF-1-M13 complexes would bind. Unbound M13 was washed away with a solution of 0.1% Tween-20 in TBS, then the phage were eluted with IGF-1, leaving the biotinylated IGF-1 bound to the Avidin on the plate. These phage were then amplified to form a secondary phage library enhanced for phage capable of binding to IGF-1. Phage from this secondary library were combined with
20 fresh K⁶⁵-Biotin-IGF-1, and these new IGF-1-M13 complexes were bound to Avidin on a tissue culture plate as described above. A more stringent wash with TBS containing 0.2% Tween-20 was performed, then an elution using IGFBP-2 elutes those phage which were capable of binding IGF-1 at a site where they could effectively compete with IGFBP-2 for binding to IGF-1. These phage were amplified, forming a library of
25 phage containing peptides that act as insulin growth factor antagonists. This second round of screening was duplicated twice again, additionally purifying the phage library for peptides capable of binding IGF-1 at a site where they could effectively compete with IGFBP-2 for binding to IGF-1. DNA from individual M13 phage were isolated and the inserted regions of their pIII genes were sequenced. These sequences were

translated and aligned to determine a consensus sequence. Some peptides selected based on their ability to compete with IGFBP-2 for binding to IGF-1 during screening of a heptapeptide library using the Phage Display technique include the amino acid sequences corresponding to SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79.

10 EXAMPLE 7

Using a second IGF-1 photoprobe, N^αGly¹(bed)-IGF-1, a biotin moiety was introduced at the site of IGF-1 insertion into IGFBP-2. The biotin moiety was transferred to IGFBP-2 upon binding to N^αGly¹(bed)-IGF-1, allowing isolation of a biotinylated peptide of IGFBP-2 from the IGF-1 binding site.

15 The photoprobe was produced by reacting Sulfo-SBED (Pierce Chemical Co., , Rockford, IL) with IGF-1 to produce N^αGly¹(bed)-IGF-1 as described above in Example 1 for the synthesis and purification of N^αGly¹AB-IGF-1, except that a ratio of 2.5 to 1 for SBED to IGF-1 was used in the present method. As with the use of HSAB, four products were eluted, which included unreacted IGF-1 (peak 1) and three reacted IGFs
20 (peaks 2-4). Peak 4 was the Gly¹ derivative N^αGly¹(bed)-IGF-1.

The photoprobe was covalently inserted into IGFBP-2 by reaction with N^αGly¹(bed)-IGF-1 (125 μg N^αGly¹(bed)-IGF-1/ 500μg IGFBP-2) using the method of Example 2. Samples were reduced and alkylated with TCEP and 4-vinylpyridene,
25 releasing the IGF-1. Aliquots of the reduced and alkylated photolabeled complex were analyzed by electrophoresis followed by immunoblotting with a polyclonal anti-IGFBP-2 antibody or by probing the blot with avidin-peroxidase. A non-reduced form of the photolabeled complex was not reactive with avidin, presumably because the biotin moiety was buried in IGFBP-2. The reduced and alkylated photolabeled

complex, having lost its IGF-1 component, migrates with an apparent mass equivalent to free IFGBP-2.

Reduced and alkylated photolabeled complex was trypsin digested and the
5 resulting biotinylated peptides were isolated by avidin affinity chromatography.
Specifically, the treated complexes were applied to a column of monomeric-avidin.
Several peaks were eluted, of which peaks 3 and 4 corresponded to biotinylated
peptides. Using MALDI-TOF-MS analysis of the monomeric-avidin column eluate,
Peak 4 was identified as having a mass of 2,595.7 Da. After correcting the observed
10 mass for the associated biotin and spacer, the peak was identified as a tryptic peptide
212-227, a C-terminal fragment of IGFBP-2.

EXAMPLE 8

To measure potential interactions between IGFBP-mimetic peptides and IGF-1,
15 BiaCore® 3000 technology (Biacore International AB, Uppsala, Sweden) was used to
allow visualization of macromolecular interactions in "real time." This instrument
utilizes a sensor chip containing four flow cells. Two of the four cells were decorated
with a specific target molecule. The other two cells served as background controls. A
sensor chip SA (Streptavidin coated) was used because the high affinity of avidin for
20 biotin allowed non-covalent attachment of biotinylated molecules to the sensor chip.
IGF-1 biotinylated on residue Lysine-65, as used in the phage display procedure above,
was immobilized, allowing optimal presentation of the IGFBP-binding domain from
the sensor chip surface. Subsequently, the potential IGFBP mimetic analytes were
added, and changes in refractive index resulting from specific interactions between the
25 analyte and the surface bound target were detected and displayed in sensorgrams. The
sensorgram for an injection of IGFBP-2 (1 µg/10 µl) is presented in Figure 15. The gray
profile represents an identical injection of analyte into the control flow cell (SA only),
providing the signal for non-specific binding and bulk flow effects. The difference
between these two signals is the specific binding of IGFBP-2 to IGF-1 (300 response

units). This approach was taken for the analysis of all of the following synthetic, amidated peptides: WHKPRPT (SEQ ID NO:76), WHKPRPR (SEQ ID NO:79), CHLFYNEQQEARGVHT (SEQ ID NO:128), ERGPLEHLYSLHIPNC (SEQ ID NO:84), and CVNPNTGKLIQGAPTI (SEQ ID NO:129). The synthetic, amidated peptides were not purified prior to the assay and were only 80-95% pure. The peptides corresponding to SEQ ID NO:76 and SEQ ID NO:79 showed significant specific binding activity. (See Figures 16 and 17, respectively.) The peptide corresponding to SEQ ID NO:84 exhibited almost the same maximal binding as IGFBP-2. (See Figures 18 and 16, respectively.) The maximal binding for the peptide corresponding to SEQ ID NO:84 was 18 μ g (100 μ M) as compared to 1 μ g (3.2 μ M) for IGFBP-2 at approximately 5.7% of the mass of IGFBP-2 (1,878 for the peptide corresponding to SEQ ID NO:84 to 31,453 Daltons for IGFBP-2.) The difference in mass may account for the lower response units observed for the peptide corresponding to SEQ ID NO:84. The peptides corresponding to SEQ ID NO:128 and SEQ ID NO:129, however, had no significant binding. (Data not shown.)

EXAMPLE 9

A peptide library is also screened using a Phage Display technique in order to identify IGFBP antagonists. A fragment of biotinylated IGFBP (e.g., the peptide of SEQ ID NO:84) is used to screen a library of randomly generated peptides for peptides capable of competing with IGF-1 for binding to IGFBP-2. The Phage Display technique as described above is modified for this screening. The peptide library of randomly generated peptides inserted into the pIII coat protein of the phage M13 is combined with a 250 ng/mL solution of IGFBP-2 fragment that is biotinylated. All phage containing peptides capable of binding IGFBP-2 fragment, including those capable of the binding IGFBP-2 fragment at a site where they can effectively compete with IGF-1 for binding, binds the free IGFBP-2 fragment, forming IGFBP-2 fragment-M13 complexes. The IGFBP-2 fragment-M13 complexes, and unbound M13, are placed on a Avidin-coated tissue culture plate, to which only the IGFBP-2

fragment-M13 complexes bind. Unbound M13 is washed away with a solution of 0.1% Tween-20 in TBS, then the phage are eluted with IGFBP-2 fragment, leaving the biotinylated IGFBP-2 fragment bound to the Avidin on the plate. These phage are then amplified to form a secondary phage library enhanced for phage capable of binding to

5 IGFBP-2 fragment. Phage from this secondary library are combined with fresh biotinylated IGFBP-2 fragment, and these new IGFBP-2 fragment-M13 complexes are bound to Avidin on a tissue culture plate and washed as described above. Those phage which are capable of binding IGFBP-2 at a site where they could effectively compete with IGF-1 for binding to IGFBP-2 are eluted with IGF-1. These phage are amplified,

10 forming a library of phage containing peptides that act as IGFBP antagonists. This second round of screening is duplicated twice and the phage library is additionally purified. DNA from individual M13 phage are isolated and the inserted regions of their pIII genes are sequenced. These sequences are translated and aligned to determine a consensus sequence. Peptides are then selected based on their ability to compete with

15 IGF-1 for binding to the IGFBP-2 fragment during screening of a heptapeptide library using the Phage Display technique.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference

20 into this application in order to more fully describe the state of the art to which this invention pertains.

Although the present process has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be

25 regarded as limitations upon the scope of the invention except as and to the extent that they are included in the accompanying claims.

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What is claimed is:

1. An isolated peptide comprising an insulin-like growth factor binding domain of an insulin-like growth factor binding protein or modification thereof, which binds insulin-like growth factor with at least about the same binding affinity as the full length insulin-like growth factor binding protein.
2. The isolated peptide of claim 1, wherein the binding domain or modification thereof binds to insulin-like growth factor-1 or insulin-like growth factor-2.
3. The isolated peptide of claim 1, wherein the binding domain is the binding domain of insulin-like growth factor binding protein-2.
4. The isolated peptide of claim 3, wherein the binding domain or modifications thereof comprises an amino acid sequence selected from the group consisting of SEQ ID NO:84, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, SEQ ID NO:92, SEQ ID NO:93, SEQ ID NO:94, SEQ ID NO:95, SEQ ID NO:96, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:101, SEQ ID NO:102, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106, SEQ ID NO:107, SEQ ID NO:108, SEQ ID NO:109, SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:130, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:134, SEQ ID NO:135, SEQ ID NO:136, SEQ ID NO:137, SEQ ID NO:138, SEQ ID NO:139, SEQ ID NO:140, and SEQ ID NO:141.
5. The isolated peptide of claim 4, wherein the amino acid sequence has at least one intrasequence disulfide bond.

6. The isolated peptide of claim 1, wherein the binding domain is the binding sequence of insulin-like growth factor binding protein-1.
7. The isolated peptide of claim 1, wherein the binding domain is the binding domain of insulin-like growth factor binding protein-3.
8. The isolated peptide of claim 1, wherein the binding domain is the binding domain of insulin-like growth factor binding protein-4.
9. The isolated peptide of claim 1, wherein the binding domain is the binding domain of insulin-like growth factor binding protein-5.
10. The isolated peptide of claim 1, wherein the binding domain is the binding domain of insulin-like growth factor binding protein-6.
11. The isolated peptide of claim 1, wherein the binding domain or modification thereof has a molecular weight of about 14 kDa or less under non-reducing conditions.
12. An insulin-like growth factor antagonist, comprising a peptide of 250 or fewer amino acids, wherein the peptide can displace bound insulin-like growth factor binding protein from insulin-like growth factor and wherein the peptide reduces binding of insulin-like growth factor to an insulin-like growth factor receptor.
13. The insulin-like growth factor antagonist of claim 12, wherein the peptide or modification thereof does not reduce binding of insulin to an insulin receptor.
14. The insulin-like growth factor antagonist of claim 12, comprising an amino acid sequence selected from the group consisting of SEQ ID NO:58, SEQ ID NO:59,

SEQ ID NO:60, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, and SEQ ID NO:79.

15. An insulin-like growth factor antagonist of claim 12, comprising an isolated peptide containing an insulin-like growth factor binding domain of an insulin-like growth factor binding protein or modification or fragment thereof, which reduces binding of insulin-like growth factor to an insulin-like growth factor receptor.
16. The insulin-like growth factor antagonist of claim 15, wherein the peptide or modification thereof does not reduce binding of insulin to an insulin receptor.
17. The insulin-like growth factor antagonist of claim 16, comprising the amino acid sequence of SEQ ID NO: 84.
18. A fragment of insulin-like growth factor, wherein the fragment binds to a binding domain on insulin-like growth factor binding protein.
19. The fragment of claim 18, wherein the insulin-like growth factor is selected from the group consisting of IGF-1 and IGF-2.
20. The fragment of claim 18, wherein the insulin-like growth factor binding protein is selected from the group consisting of insulin-like growth factor binding protein-1, insulin-like growth factor binding protein-2, insulin-like growth factor binding protein-3, insulin-like growth factor binding protein-

4, insulin-like growth factor binding protein-5, and insulin-like growth factor binding protein-6.

21. The fragment of claim 18, wherein the fragment reduces binding of insulin-like growth factor to insulin-like growth factor binding protein.
22. The fragment of claim 18, comprising the amino acid sequence of SEQ ID NO:80.
23. The fragment of claim 2, further comprising the amino acid sequence of SEQ ID NO:82 linked to SEQ ID NO:80 by a disulfide bond.
24. A protein complex, comprising the peptide of claim 1 and a fragment of insulin-like growth factor, wherein the fragment binds to a binding domain on insulin-like growth factor binding protein.
25. The protein complex of claim 24, wherein the IGF fragment is a photoaffinity derivative of an insulin-like growth factor.
26. The protein complex of claim 25, wherein the photoaffinity derivative comprises a photoreactive glycine residue within an insulin-like growth factor binding protein binding domain of insulin-like growth factor.
27. The protein complex of claim 26, wherein the photoreactive glycine residue is an N-terminal glycine residue.
28. The protein complex of claim 27, wherein the N-terminal derivative is N^αGly¹(4-azidobenzoyl)-insulin-like growth factor-1.

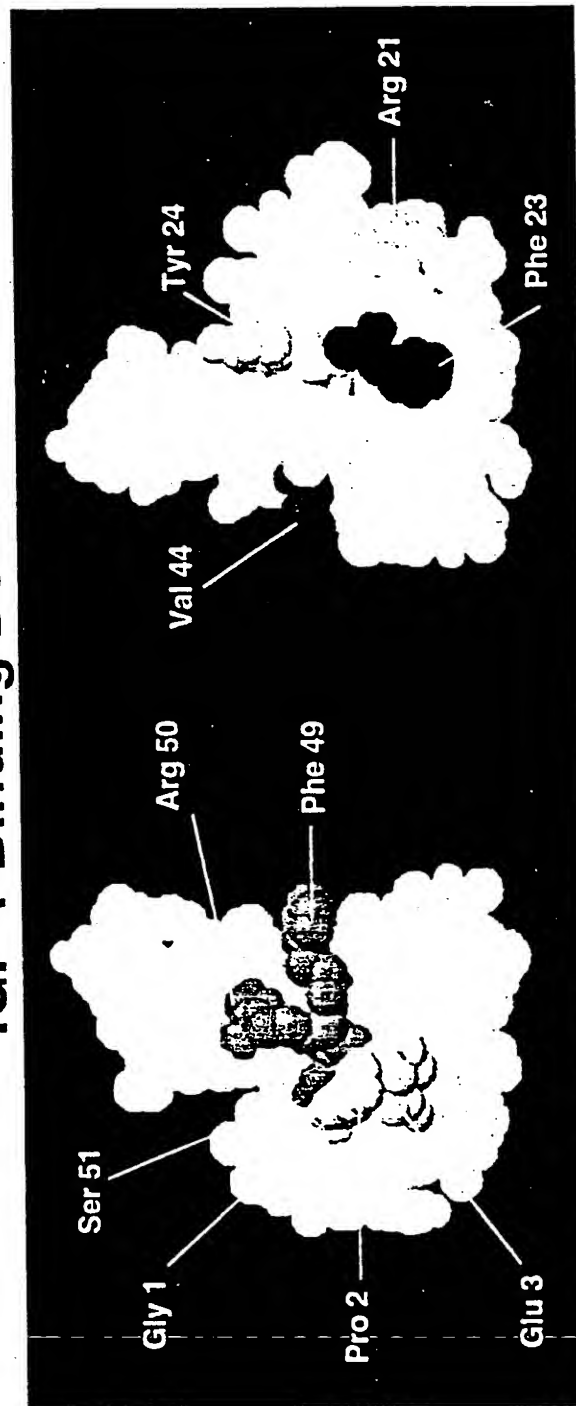
29. A method of treating a subject with cancer or of preventing cancer in a subject, comprising administering to the subject the insulin-like growth factor antagonist of claim 12.
30. The method claim 29, wherein the cancer comprises a tumor that expresses an insulin-like growth factor receptor.
31. The method of claim 30, wherein the cancer is breast cancer.
32. The method of claims 30, wherein the cancer is prostate cancer.
33. A method of treating a subject with a diabetic complication exacerbated by insulin-like growth factor or of preventing a diabetic complication exacerbated by insulin-like growth factor in a subject, comprising administering to the subject the antagonist of claim 12.
34. The method of claim 33, wherein the diabetic complication is diabetic retinopathy.
35. The method of claim 33, wherein the diabetic complication is diabetic nephropathy.
36. A method of treating a subject with cancer or of preventing cancer in a subject, comprising administering to the subject the insulin-like growth factor antagonist of claim 15.
37. The method claim 36, wherein the cancer comprises a tumor that expresses an insulin-like growth factor receptor.

38. The method of claim 37, wherein the cancer is breast cancer.
39. The method of claims 37, wherein the cancer is prostate cancer.
40. A method of treating a subject with a diabetic complication exacerbated by insulin-like growth factor or of preventing a diabetic complication exacerbated by insulin-like growth factor in a subject, comprising administering to the subject the antagonist of claim 15.
41. The method of claim 40, wherein the diabetic complication is diabetic retinopathy.
42. The method of claim 40, wherein the diabetic complication is diabetic nephropathy.
43. A method of treating a subject with an ischemic injury, comprising administering to the subject the fragment of claim 21, thereby reducing the ischemic injury in the subject.
44. A method of treating a subject with diabetes, comprising administering to the subject the fragment of claim 21, thereby reducing blood glucose levels in the subject.
45. A nucleic acid that encodes the peptide of claim 1.
46. A vector for the expression of an insulin-like growth factor binding domain of an insulin-like growth factor binding protein or modification thereof, which binds insulin-like growth factor with at least about the same binding affinity as

the full length insulin-like growth factor binding protein, comprising the nucleic acid of claim 45.

47. A cell containing an exogenous nucleic acid comprising the nucleic acid of claim 45.
48. An isolated IGF-1, wherein one residue of the IGF-1 is biotinylated, and wherein the biotinylated IGF-1 has an affinity for IGFBP-2 of about 300 to 50 pM in a competition binding assay.
49. The isolated IGF-1 of claim 48, wherein the IGF-1 is selected from the group consisting of N^αGly¹-, N^εLys⁶⁵-, and N^εLys²⁷- monobiotinylated IGF-1.

IGF-1 Binding Domains



IGF-1R-Binding Domain

IGFBP-Binding Domain

FIG. 1

IGF-1 STRUCTURE

B	Human IGF-1	1	G	P	E	T	L	C	G	A	E	L	V	D	A	L	Q	F	V	C	G	A	R	G	F	Y	F	N	K	P	T	...	29			
	IGF-2		A	Y	R	P	S	E	T	L	C	G	G	E	L	V	D	T	L	N	F	V	C	G	D	R	G	F	Y	F	S	R	P	A	...	25
	Porcine Insulin				F	V	N	Q	H	L	C	G	S	H	L	V	E	A	L	Y	L	V	C	G	E	R	G	F	F	Y	T	P	K	A		

C		30								35					40																				
	IGF-1		G	Y	G	S	S	S	R	R	A	P	N	T																					
	IGF-2		S	-	-	R	V	S	R	R	S	R	-	-																					

A	IGF-1	42	G	I	V	D	E	E	C	C	F	R	S	C	D	L	R	R	L	E	M	Y	C	A	...									
	IGF-2		G	I	V	E	E	C	C	C	F	R	S	C	D	L	A	L	L	E	T	Y	C	A	...									
	Insulin		G	I	V	E	E	Q	C	C	C	T	S	I	C	S	L	Y	Q	L	E	N	Y	C	N									

D		63																																		
	IGF-1		P	L	K	P	A	K	S	A																										
	IGF-2		T	-	-	P	A	K	S	E																										

FIG.2

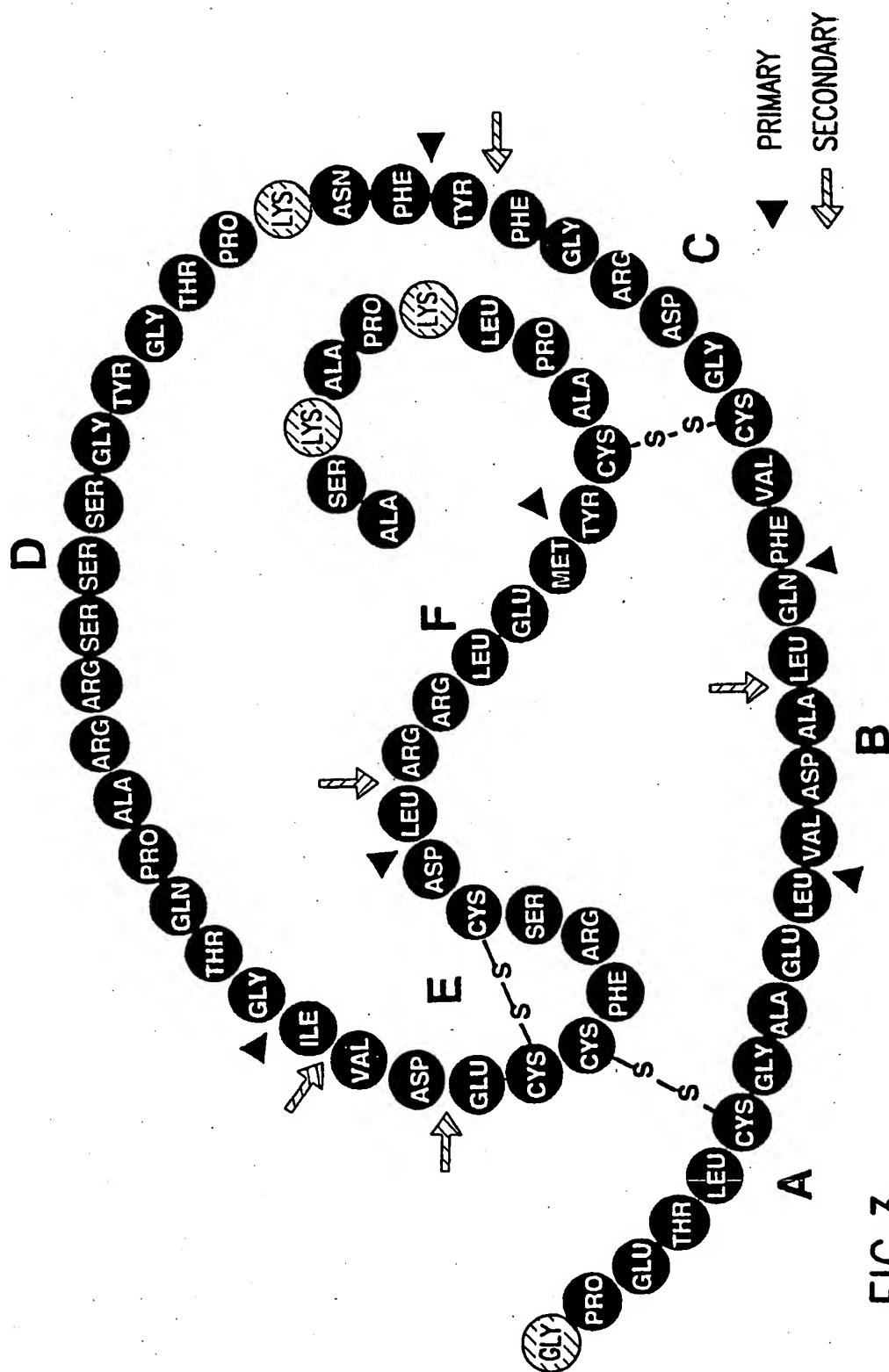


FIG. 3

IGFBP-1 ~~~~~AP WQCAPCSAEK LALCPPVSAS CSEVT RSAGCGCCPM
 IGFBP-2 ~~~~~EVL FRCPCTPER LAACPPPPVA PPAVAAVAG GARMPCAEV REPCCGCCSV
 IGFBP-3 GASSGGLGPV VRCEPCDARA LAQCAPPVAV CAELV REPCCGCCLT
 IGFBP-4 ~~~~~DEA IHCPCCSEK LARCRP.PVG CEELV REPCCGCCAT
 IGFBP-5 ~~~~~LGSF VHCEPCDEKA LSMCPPSPLG C.ELV KEPCCGCCMT
 IGFBP-6 ~~~~~AL ARCPGCCQGV QAGCPGGCVE EEDGGS PAEGCAEAE

IGFBP-1 CALPLGAACG VATARCARGL SCRALPGEQQ PLHALTRGQG ACVQESDAS. ..APHAAEAG
 IGFBP-2 CARLEGEACG VYTPROGQGL RCYPHPGSEL PLQALVMGEG TCEKRRDAEY GASPEQVADN
 IGFBP-3 CALSEGQPCG IYTERCGSGL RCQPSPEAR PLQALLDGRG LCVNASAVSR LRAYLLPAPP
 IGFBP-4 CALGLMPCG VYTPROGQGL RCYPPRGVEK PLHTLMHGQG VCMELAEIE. ..AIQESLQP
 IGFBP-5 CALAEGQSCG VYTERCAQGL RCLPRQDEEK PLHALLHGRG VCLNEKSYRE QVK
 IGFBP-6 CLRREGQECG VYTPNCAPGL QCHPPKDEA PLRALLLGRG RCLPARA... PA

IGFBP-1 SPESPESTEI TEEE..... ..LLDNFH LMAPSEEDHS ILWDAISTYD GSKALHVTNI
 IGFBP-2 GDDHSEGLV ENHVDSTMM LGGGGSAGRK PLKSGMKELA VFREKVTEQH RQMGKGGKH
 IGFBP-3 APGNASEEE DRSAGSVESV SVSSTHRVSD PKFHPLHSKI I I I KGHAKD SQRYKVDYES
 IGFBP-4 S.DKDEGDHP NNSF..... ..SPCSAH DRRCLQKHFA KIRDRSTSGG KMKVNGAPRE
 IGFBP-5 IERDSREHEE PTTSEMAEET YSPKIFRPKH TRISELKAEA VKKDRRKKLT QSKFVGGAEN
 IGFBP-6 VAEENPKESK PQAGTARPQD VNR..... ..RDQQR....NPG

IGFBP-1 KKWKE...P. CRIELRVVE SLAKAQETSGEEISKF YLPNCNKNKF
 IGFBP-2 LGLEE...PK KLRPPPARTP CQQLDQVLE RISTMRPDE RGPLEHLYSL HIPNCDKHGL
 IGFBP-3 QSTDTONFSS ESKRETEYGP CRREMEDTLN HLKFLNLVSP RG.....V HIPNCDKKGF
 IGFBP-4 DARPV...P.QGS CQSELHRALE RLAASQSRTHEDLYII PIPNCDRNGN
 IGFBP-5 TAHPRIISAP EMRQESQGP CRRHMEASLQ ELKASPRMVP RA.....V YLPNCDRKGF
 IGFBP-6 TSTTPSQNS AGVQDTEMGP CRRHLDVLQ QLQT..... .EVYGAQTL YVPNCDHRGF

IGFBP-1 YHSRQCETSM DGEAGLCWCV YPWNGKRIPG SPEIRGDPNC QIYFNVQN ~~~~~
 IGFBP-2 YNLKQCKMSL NGQRGECWCV NPNTGKLIQG APTIRGDPNC HLFYNEQQA RGVHTQRMQ
 IGFBP-3 YKKKQCRPSK GRKRGCWCV DKYQPLPGY TTKGKEDVHC YSMQSK ~~~~~
 IGFBP-4 FHPKQCHPAL DGQRGKCWCV DRKTGVKLPG GLEPKGELDC HQLADSFRE ~~~~~
 IGFBP-5 YKRKQCKPSR GRKRGCWCV DKYGMKLPGM EYV.DGDFQC HTFDSSNE ~~~~~
 IGFBP-6 YRKRQCRSSQ GRRGRCWCV DRM.GKSLPG SPDGNGSSSC PTGSSG ~~~~~

FIG.4

MALDI ANALYSIS OF 15.8 kDa IGFBP-2 FRAGMENT

1 EVLFRCPPCTPERLAACGPPPVAPPAAVAAGGARMPCAELVREPGCGC
51 CSVCARLEGEACGVYTPRCGQGLRCYPHPGSELPLQALVMGEGTCEKRRD
101 AEYGASPEQVADNGDDHSEGLVENHVDSTMNMLGGGGSAGRKPLKSGMK
151 ELAVFREKVTEQHRQMGKGKHHLGLEEPKKLRPPPARTPCQQELDQVLE
201 RISTMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCV
251 NPNTGKLIQGAPTIRGDPECHLFYNEQQEACGVHTQRMQ

FIG.5

212 GPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCV 250
251 NPNTGKLIQGAPTIRGDPECHLFYNEQQEACGVHTQR 287

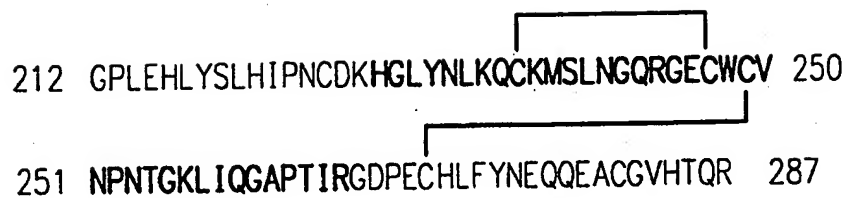
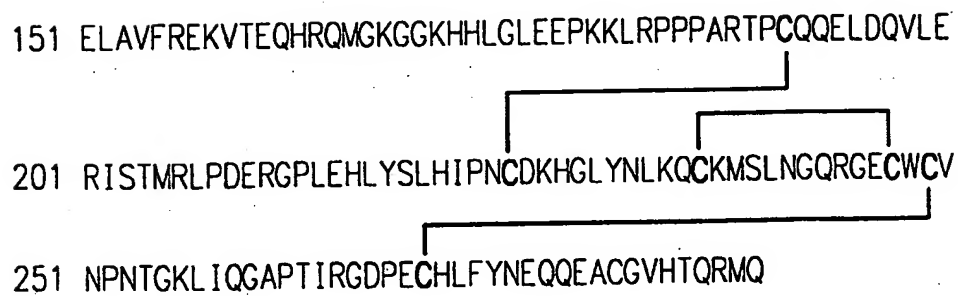


FIG.6

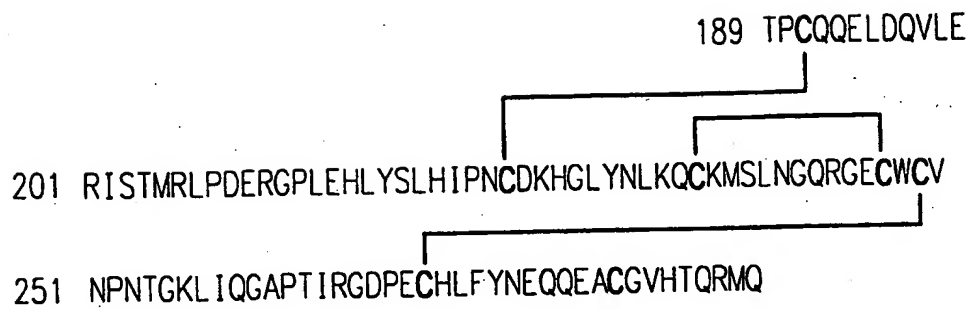
151 ELAVFREKVTEQHRQMKGKGGKHHLGLEEPKKLRPPPARTPCQQELDQVLE
201 RISTMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCKMSLNGQRGECWCV
251 NPNTGKLIQGAPTIRGDPECHLFYNEQQEACGVHTQRMQ



IGF BINDING DOMAIN STRUCTURE
RESIDUES 151-289

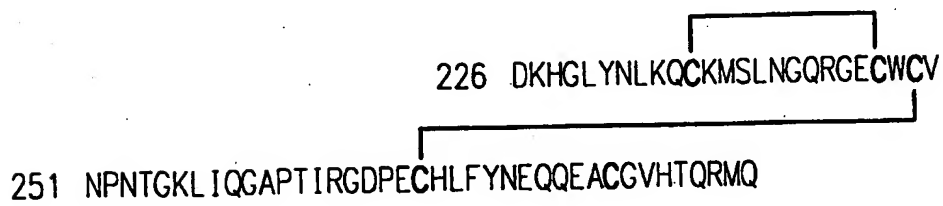
FIG.7

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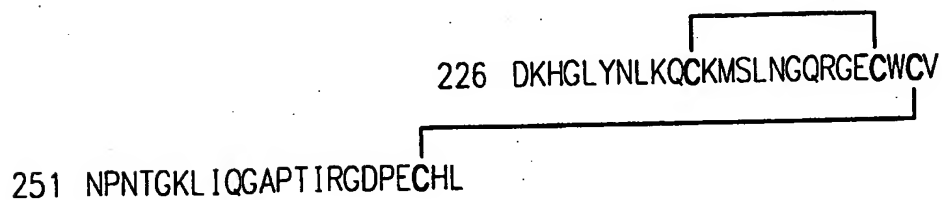
IGF BINDING DOMAIN STRUCTURE
RESIDUES 189-289

FIG.8



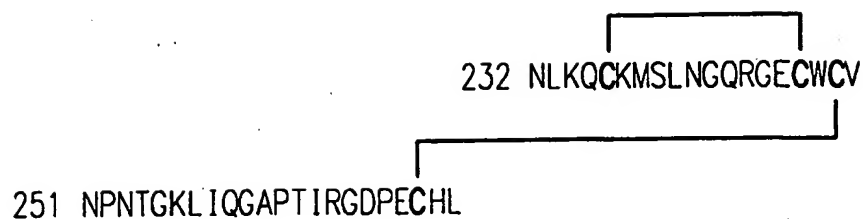
IGF BINDING DOMAIN STRUCTURE
RESIDUES 226-289

FIG.9



IGF BINDING DOMAIN STRUCTURE
RESIDUES 226-272

FIG.10



IGF BINDING DOMAIN STRUCTURE
RESIDUES 232-272

FIG.11



IGF BINDING DOMAIN STRUCTURE 6
DIMERIZED MOTIF OF 232-249 AND 236-248.
UNDERLINED RESIDUES ARE REPEATED IN THE STRUCTURE

FIG.12



IGF BINDING DOMAIN STRUCTURE 7
REPEATED MOTIF OF RESIDUES 248-270 AND 248-272.

FIG.13

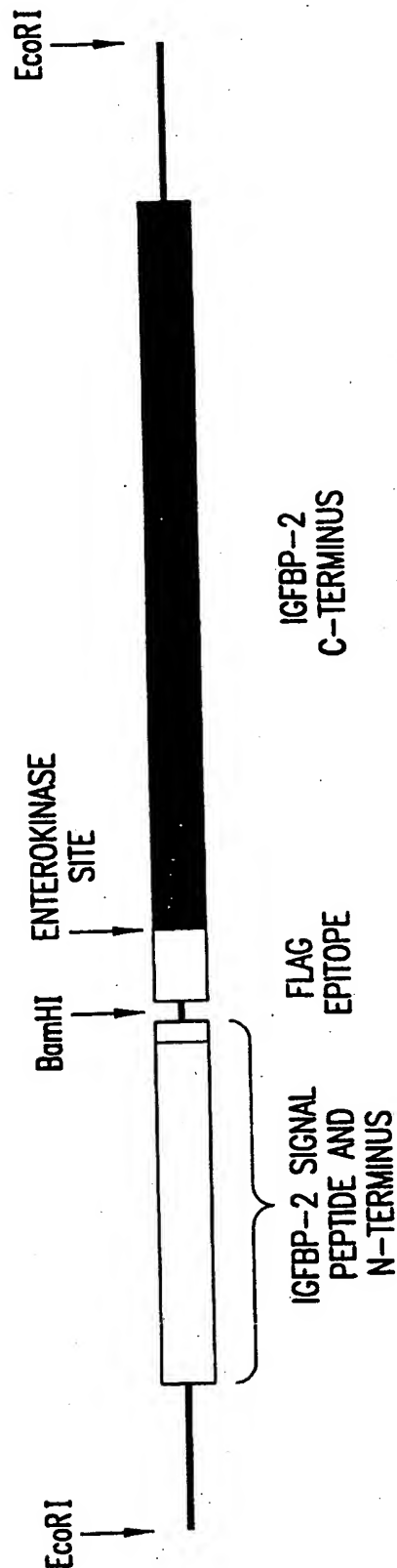


FIG.14

10 / 11

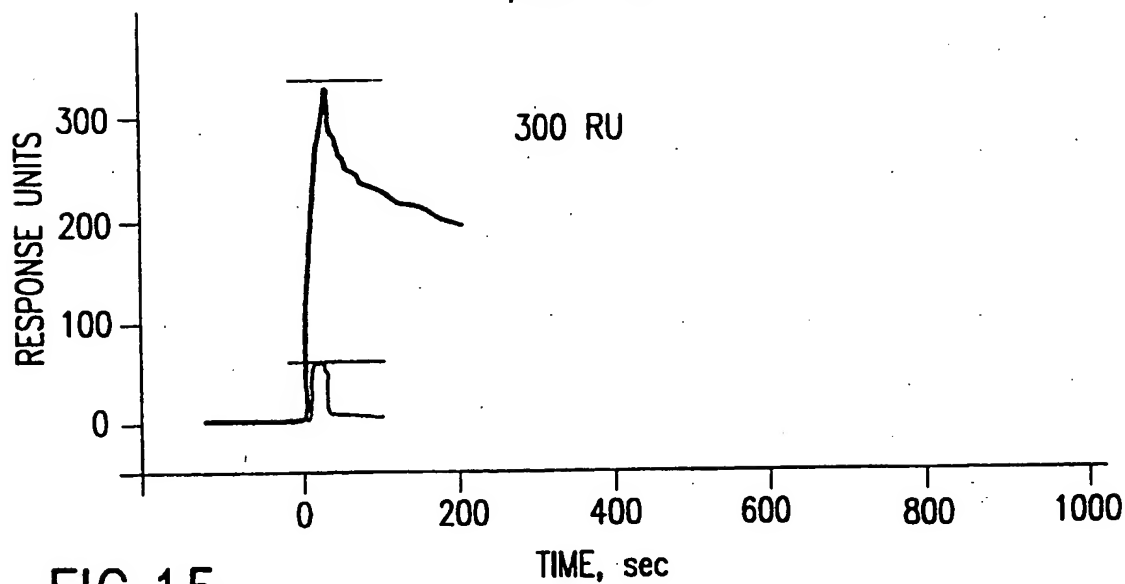
ANALYTE: IGFBP-2
31,453.7 Da

FIG.15

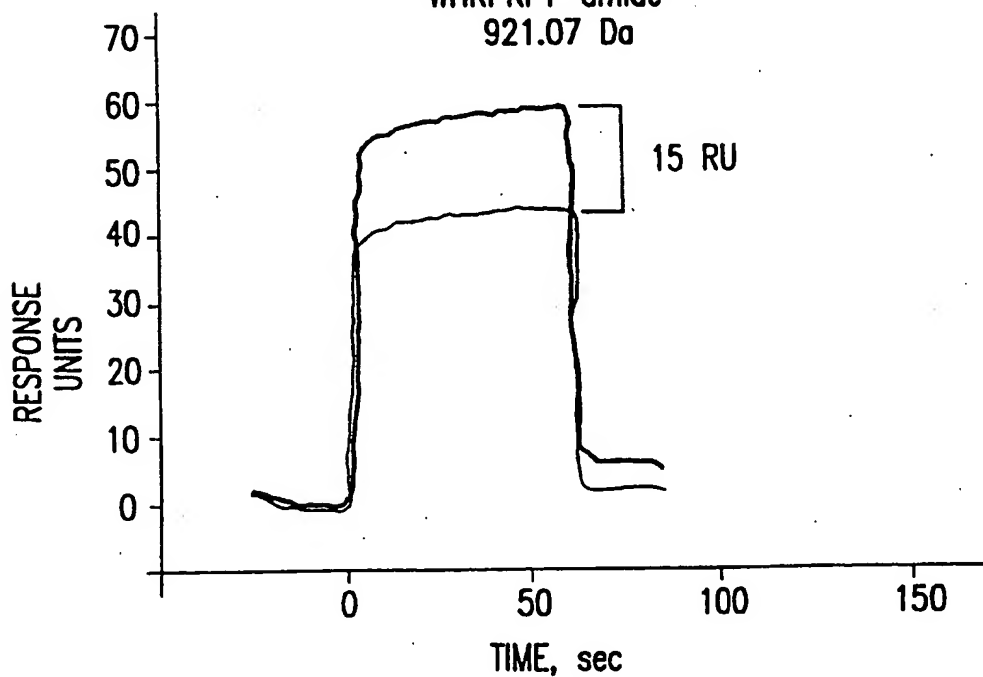
ANALYTE: mime-2
WHKPRPT-amide
921.07 Da

FIG.16

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ANALYTE: mime-3
WHKPRPR-amide
976.15 Da

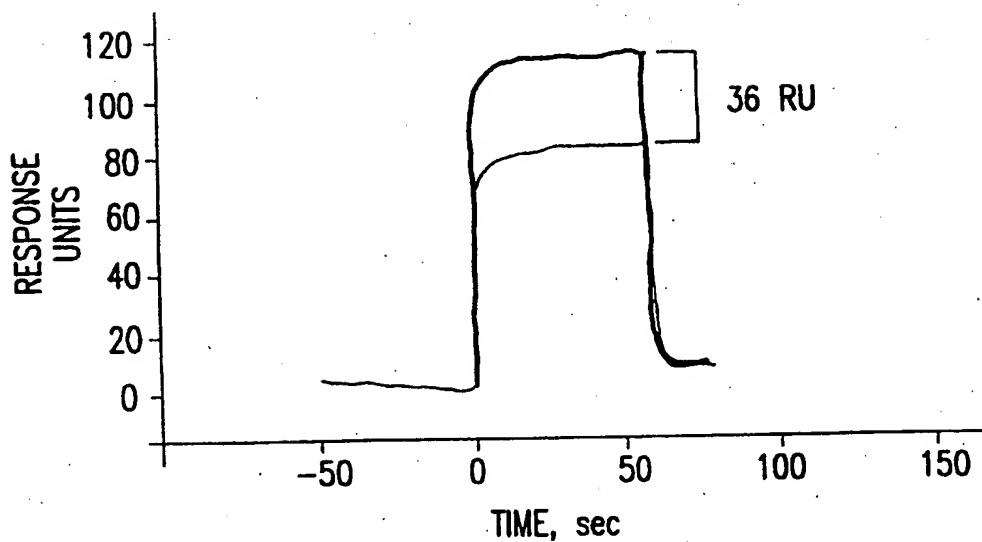


FIG.17

ANALYTE: PLP-2
ERGPLEHLYSLHIPNC-amide
1,878.14 Da

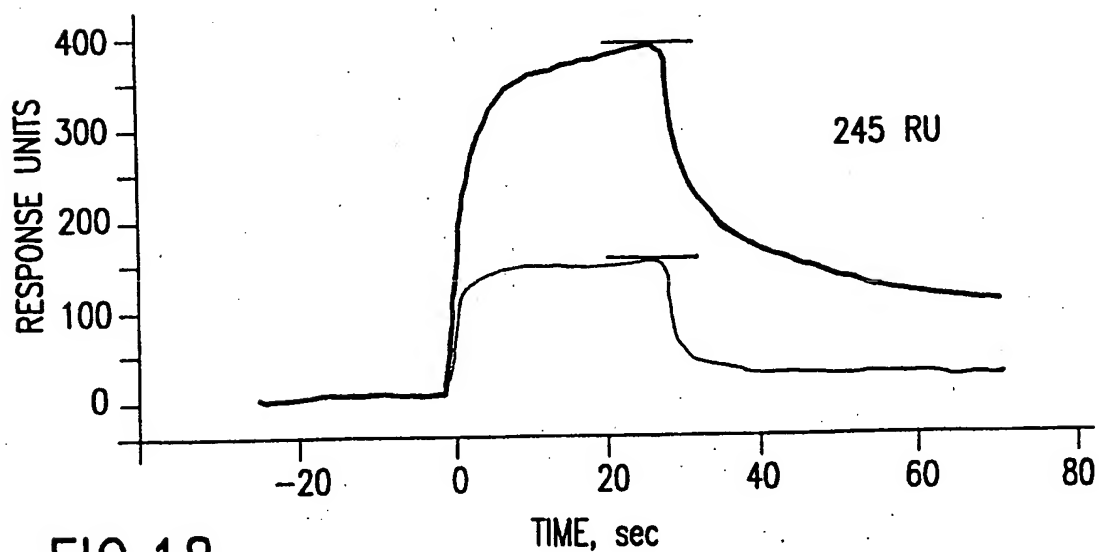


FIG.18

SEQUENCE LISTING

<110> MUSC Foundation for Research Development

Rosenzweig, Steven A.
Horney, Mark J.<120> FRAGMENTS OF INSULIN-LIKE GROWTH FACTOR
BINDING PROTEIN AND INSULIN-LIKE GROWTH FACTOR AND USES
THEREOF

<130> 19113.0072/P

<150> 60/104,528

<151> 1998-10-16

<160> 142

<170> FastSEQ for Windows Version 4.0

<210> 1

<211> 67

<212> PRT

<213> Homo sapiens

<400> 1

Gly	Pro	Leu	Glu	His	Leu	Tyr	Ser	Leu	His	Ile	Pro	Asn	Cys	Asp	Lys
1				5					10					15	
His	Gly	Leu	Tyr	Asn	Leu	Lys	Gln	Cys	Lys	Met	Ser	Leu	Asn	Gly	Gln
		20						25					30		
Arg	Gly	Glu	Cys	Trp	Cys	Val	Asn	Pro	Asn	Thr	Gly	Lys	Leu	Ile	Gln
		35					40					45			
Gly	Ala	Pro	Thr	Ile	Arg	Gly	Asp	Pro	Glu	Cys	His	Leu	Phe	Tyr	Asn
		50				55						60			
Glu	Gln	Arg													
65															

<210> 2

<211> 66

<212> PRT

<213> Homo sapiens

<400> 2

Gly	Pro	Leu	Glu	His	Leu	Tyr	Ser	Leu	His	Ile	Pro	Asn	Cys	Asp	Lys
1				5					10					15	
His	Gly	Leu	Tyr	Asn	Leu	Lys	Gln	Cys	Lys	Met	Ser	Leu	Asn	Gly	Gln
		20						25					30		
Arg	Gly	Glu	Cys	Trp	Cys	Val	Asn	Pro	Asn	Thr	Gly	Lys	Leu	Ile	Gln
		35					40					45			
Gly	Ala	Pro	Thr	Ile	Arg	Gly	Asp	Pro	Glu	Cys	His	Leu	Phe	Tyr	Asn
		50				55						60			
Glu	Gln														
65															

<210> 3

<211> 66

<212> PRT

<213> Homo sapiens

<400> 3

```

Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His
 1           5           10           15
Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg
          20           25           30
Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly
          35           40           45
Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu
          50           55           60
Gln Arg
65

```

<210> 4

<211> 65

<212> PRT

<213> Homo sapiens

<400> 4

```

Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His
 1           5           10           15
Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg
          20           25           30
Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly
          35           40           45
Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu
          50           55           60
Gln
65

```

<210> 5

<211> 65

<212> PRT

<213> Homo sapiens

<400> 5

```

Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly
 1           5           10           15
Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly
          20           25           30
Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala
          35           40           45
Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
          50           55           60
Arg
65

```

<210> 6

<211> 64

<212> PRT

<213> Homo sapiens

<400> 6

```

Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly
 1           5           10           15
Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly
          20           25           30

```

Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala
 35 40 45
 Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
 50 55 60

<210> 7

<211> 64

<212> PRT

<213> Homo sapiens

<400> 7

Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu
 1 5 10 15
 Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu
 20 25 30
 Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro
 35 40 45
 Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln Arg
 50 55 60

<210> 8

<211> 63

<212> PRT

<213> Homo sapiens

<400> 8

Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu
 1 5 10 15
 Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu
 20 25 30
 Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro
 35 40 45
 Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
 50 55 60

<210> 9

<211> 63

<212> PRT

<213> Homo sapiens

<400> 9

His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr
 1 5 10 15
 Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys
 20 25 30
 Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr
 35 40 45
 Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln Arg
 50 55 60

<210> 10

<211> 62

<212> PRT

<213> Homo sapiens

<400> 10

His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr
 1 5 10 15

4

```

Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys
      20                      25                      30
Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr
      35                      40                      45
Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
      50                      55                      60

```

<210> 11
 <211> 62
 <212> PRT
 <213> Homo sapiens

```

<400> 11
Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn
 1                      5                      10                      15
Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp
      20                      25                      30
Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile
      35                      40                      45
Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln Arg
      50                      55                      60

```

<210> 12
 <211> 61
 <212> PRT
 <213> Homo sapiens

```

<400> 12
Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn
 1                      5                      10                      15
Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp
      20                      25                      30
Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile
      35                      40                      45
Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
      50                      55                      60

```

<210> 13
 <211> 61
 <212> PRT
 <213> Homo sapiens

```

<400> 13
Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu
 1                      5                      10                      15
Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys
      20                      25                      30
Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg
      35                      40                      45
Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln Arg
      50                      55                      60

```

<210> 14
 <211> 60
 <212> PRT
 <213> Homo sapiens

<400> 14

5

```

Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu
 1           5           10           15
Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys
          20           25           30
Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg
      35           40           45
Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
 50           55           60

```

<210> 15
 <211> 60
 <212> PRT
 <213> Homo sapiens

```

<400> 15
Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys
 1           5           10           15
Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val
          20           25           30
Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly
      35           40           45
Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln Arg
 50           55           60

```

<210> 16
 <211> 59
 <212> PRT
 <213> Homo sapiens

```

<400> 16
Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys
 1           5           10           15
Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val
          20           25           30
Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly
      35           40           45
Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
 50           55

```

<210> 17
 <211> 59
 <212> PRT
 <213> Homo sapiens

```

<400> 17
Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln
 1           5           10           15
Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn
          20           25           30
Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp
      35           40           45
Pro Glu Cys His Leu Phe Tyr Asn Glu Gln Arg
 50           55

```

<210> 18
 <211> 58
 <212> PRT
 <213> Homo sapiens

<400> 18

Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln
 1 5 10 15
 Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn
 20 25 30
 Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp
 35 40 45
 Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
 50 55

<210> 19

<211> 58

<212> PRT

<213> Homo sapiens

<400> 19

His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys
 1 5 10 15
 Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro
 20 25 30
 Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro
 35 40 45
 Glu Cys His Leu Phe Tyr Asn Glu Gln Arg
 50 55

<210> 20

<211> 57

<212> PRT

<213> Homo sapiens

<400> 20

His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys
 1 5 10 15
 Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro
 20 25 30
 Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro
 35 40 45
 Glu Cys His Leu Phe Tyr Asn Glu Gln
 50 55

<210> 21

<211> 57

<212> PRT

<213> Homo sapiens

<400> 21

Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys
 1 5 10 15
 Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn
 20 25 30
 Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu
 35 40 45
 Cys His Leu Phe Tyr Asn Glu Gln Arg
 50 55

<210> 22

<211> 56

<212> PRT

<213> Homo sapiens

<400> 22

```

Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys
 1              5              10              15
Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn
              20              25              30
Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu
              35              40              45
Cys His Leu Phe Tyr Asn Glu Gln
      50              55

```

<210> 23

<211> 56

<212> PRT

<213> Homo sapiens

<400> 23

```

Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met
 1              5              10              15
Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr
              20              25              30
Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys
              35              40              45
His Leu Phe Tyr Asn Glu Gln Arg
      50              55

```

<210> 24

<211> 55

<212> PRT

<213> Homo sapiens

<400> 24

```

Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met
 1              5              10              15
Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr
              20              25              30
Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys
              35              40              45
His Leu Phe Tyr Asn Glu Gln
      50              55

```

<210> 25

<211> 55

<212> PRT

<213> Homo sapiens

<400> 25

```

Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser
 1              5              10              15
Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly
              20              25              30
Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His
              35              40              45
Leu Phe Tyr Asn Glu Gln Arg
      50              55

```

<210> 26

<211> 54
 <212> PRT
 <213> Homo sapiens

<400> 26
 Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser
 1 5 10 15
 Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly
 20 25 30
 Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His
 35 40 45
 Leu Phe Tyr Asn Glu Gln
 50

<210> 27
 <211> 54
 <212> PRT
 <213> Homo sapiens

<400> 27
 Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu
 1 5 10 15
 Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys
 20 25 30
 Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu
 35 40 45
 Phe Tyr Asn Glu Gln Arg
 50

<210> 28
 <211> 53
 <212> PRT
 <213> Homo sapiens

<400> 28
 Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu
 1 5 10 15
 Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys
 20 25 30
 Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu
 35 40 45
 Phe Tyr Asn Glu Gln
 50

<210> 29
 <211> 52
 <212> PRT
 <213> Homo sapiens

<400> 29
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 1 5 10 15
 Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu
 20 25 30
 Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe
 35 40 45
 Tyr Asn Glu Gln
 50

<210> 30
 <211> 55
 <212> PRT
 <213> Homo sapiens

<400> 30
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 1 5 10 15
 Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu
 20 25 30
 Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe
 35 40 45
 Tyr Asn Glu Gln Gln Glu Ala
 50 55

<210> 31
 <211> 57
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (0)...(0)
 <223> Xaa can be Cys or Arg

<400> 31
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 1 5 10 15
 Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu
 20 25 30
 Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe
 35 40 45
 Tyr Asn Glu Gln Gln Glu Ala Xaa Gly
 50 55

<210> 32
 <211> 59
 <212> PRT
 <213> Homo sapiens

<220>
 <221> VARIANT
 <222> (0)...(0)
 <223> Xaa can be Cys or Arg

<400> 32
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 1 5 10 15
 Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu
 20 25 30
 Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe
 35 40 45
 Tyr Asn Glu Gln Gln Glu Ala Xaa Gly Val His
 50 55

<210> 33
 <211> 61
 <212> PRT

10

<213> Homo sapiens

<220>

<221> VARIANT

<222> (0)...(0)

<223> Xaa can be Cys or Arg

<400> 33

```

Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 1           5           10           15
Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu
           20           25           30
Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe
           35           40           45
Tyr Asn Glu Gln Gln Glu Ala Xaa Gly Val His Thr Gln
 50           55           60

```

<210> 34

<211> 64

<212> PRT

<213> Homo sapiens

<220>

<221> VARIANT

<222> (0)...(0)

<223> Xaa can be Cys or Arg

<400> 34

```

Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 1           5           10           15
Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu
           20           25           30
Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe
           35           40           45
Tyr Asn Glu Gln Gln Glu Ala Xaa Gly Val His Thr Gln Arg Met Gln
 50           55           60

```

<210> 35

<211> 139

<212> PRT

<213> Homo sapiens

<400> 35

```

Glu Leu Ala Val Phe Arg Glu Lys Val Thr Glu Gln His Arg Gln Met
 1           5           10           15
Gly Lys Gly Gly Lys His His Leu Gly Leu Glu Glu Pro Lys Lys Leu
           20           25           30
Arg Pro Pro Pro Ala Arg Thr Pro Cys Gln Gln Glu Leu Asp Gln Val
           35           40           45
Leu Glu Arg Ile Ser Thr Met Arg Leu Pro Asp Glu Arg Gly Pro Leu
           50           55           60
Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys His Gly Leu
           65           70           75           80
Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu
           85           90           95
Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro
           100           105           110

```

11

Thr Ile Arg Gly Cys Pro Glu Cys His Leu Phe Tyr Asn Glu Gln Gln
 115 120 125

Glu Ala Cys Gly Val His Thr Gln Arg Met Gln
 130 135

<210> 36
 <211> 101
 <212> PRT
 <213> Homo sapiens

<400> 36
 Thr Pro Cys Gln Gln Glu Leu Asp Gln Val Leu Glu Arg Ile Ser Thr
 1 5 10 15
 Met Arg Leu Pro Asp Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu
 20 25 30
 His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys
 35 40 45
 Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro
 50 55 60
 Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro
 65 70 75 80
 Glu Cys His Leu Phe Tyr Asn Glu Gln Gln Glu Ala Cys Gly Phe His
 85 90 95
 Thr Gln Arg Met Gln
 100

<210> 37
 <211> 47
 <212> PRT
 <213> Homo sapiens

<400> 37
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 1 5 10 15
 Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu
 20 25 30
 Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu
 35 40 45

<210> 38
 <211> 41
 <212> PRT
 <213> Homo sapiens

<400> 38
 Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys
 1 5 10 15
 Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr
 20 25 30
 Ile Arg Gly Asp Pro Glu Cys His Leu
 35 40

<210> 39
 <211> 56
 <212> PRT
 <213> Artificial Sequence

12

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 39

```

Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys
 1             5             10             15
Trp Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Lys
          20             25             30
Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Lys Met Ser Leu
          35             40             45
Asn Gly Gln Arg Gly Glu Cys Trp
 50             55

```

<210> 40

<211> 94

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 40

```

Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr
 1             5             10             15
Ile Arg Gly Asp Pro Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys
          20             25             30
Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys Trp Cys
          35             40             45
Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg
          50             55             60
Gly Asp Pro Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile
          65             70             75             80
Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu
          85             90

```

<210> 41

<211> 14

<212> PRT

<213> Homo sapiens

<400> 41

```

Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys
 1             5             10

```

<210> 42

<211> 8

<212> PRT

<213> Homo sapiens

<400> 42

```

Cys His Leu Phe Tyr Asn Glu Gln
 1             5

```

<210> 43

<211> 52

<212> PRT

<213> Homo sapiens

<400> 43

```

Asn Lys Asn Gly Phe Tyr His Ser Arg Gln Cys Glu Thr Ser Met Asp
 1             5             10             15
Gly Glu Ala Gly Leu Cys Trp Cys Val Tyr Pro Trp Asn Gly Lys Arg
          20             25             30
Ile Pro Gly Ser Pro Glu Ile Arg Gly Asp Pro Asn Cys Gln Ile Tyr
          35             40             45
Phe Asn Val Gln
          50

```

<210> 44

<211> 51

<212> PRT

<213> Homo sapiens

<400> 44

```

Asp Lys Lys Gly Phe Tyr Lys Lys Lys Gln Cys Arg Pro Ser Lys Gly
 1             5             10             15
Arg Lys Arg Gly Phe Cys Trp Cys Val Asp Lys Tyr Gly Gln Pro Leu
          20             25             30
Pro Gly Tyr Thr Thr Lys Gly Lys Glu Asp Val His Cys Tyr Ser Met
          35             40             45
Gln Ser Lys
          50

```

<210> 45

<211> 54

<212> PRT

<213> Homo sapiens

<400> 45

```

Asp Arg Asn Gly Asn Phe His Pro Lys Gln Cys His Pro Ala Leu Asp
 1             5             10             15
Gly Gln Arg Gly Lys Cys Trp Cys Val Asp Arg Lys Thr Gly Val Lys
          20             25             30
Leu Pro Gly Gly Leu Glu Pro Lys Gly Glu Leu Asp Cys His Gln Leu
          35             40             45
Ala Asp Ser Phe Arg Glu
          50

```

<210> 46

<211> 53

<212> PRT

<213> Homo sapiens

<400> 46

```

Asp Arg Lys Gly Phe Tyr Lys Arg Lys Gln Cys Lys Pro Ser Arg Gly
 1             5             10             15
Arg Lys Arg Gly Ile Cys Trp Cys Val Asp Lys Tyr Gly Met Lys Leu
          20             25             30
Pro Gly Met Glu Tyr Val Asp Gly Asp Phe Gln Cys His Thr Phe Asp
          35             40             45
Ser Ser Asn Val Glu
          50

```

14

<210> 47

<211> 50

<212> PRT

<213> Homo sapiens

<400> 47

Asp His Arg Gly Phe Tyr Arg Lys Arg Gln Cys Arg Ser Ser Gln Gly
1 5 10 15
Gln Arg Arg Gly Pro Cys Trp Cys Val Asp Arg Met Gly Lys Ser Leu
20 25 30
Pro Gly Ser Pro Asp Gly Asn Gly Ser Ser Ser Cys Pro Thr Gly Ser
35 40 45
Ser Gly
50

<210> 48

<211> 5

<212> PRT

<213> Homo sapiens

<400> 48

Phe Tyr Asn Glu Gln
1 5

<210> 49

<211> 10

<212> PRT

<213> Homo sapiens

<400> 49

Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
1 5 10

<210> 50

<211> 15

<212> PRT

<213> Homo sapiens

<400> 50

Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
1 5 10 15

<210> 51

<211> 20

<212> PRT

<213> Homo sapiens

<400> 51

Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe
1 5 10 15
Tyr Asn Glu Gln
20

<210> 52

<211> 25

<212> PRT

<213> Homo sapiens

15

<400> 52

Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro
 1 5 10 15
 Glu Cys His Leu Phe Tyr Asn Glu Gln
 20 25

<210> 53

<211> 30

<212> PRT

<213> Homo sapiens

<400> 53

Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr
 1 5 10 15
 Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
 20 25 30

<210> 54

<211> 35

<212> PRT

<213> Homo sapiens

<400> 54

Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile
 1 5 10 15
 Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr
 20 25 30
 Asn Glu Gln
 35

<210> 55

<211> 40

<212> PRT

<213> Homo sapiens

<400> 55

Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn Pro Asn
 1 5 10 15
 Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu
 20 25 30
 Cys His Leu Phe Tyr Asn Glu Gln
 35 40

<210> 56

<211> 45

<212> PRT

<213> Homo sapiens

<400> 56

Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp
 1 5 10 15
 Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly Ala Pro Thr Ile
 20 25 30
 Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu Gln
 35 40 45

<210> 57

<211> 49

<212> PRT

<213> Homo sapiens

<400> 57

Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln Arg
1 5 10 15
Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln Gly
20 25 30
Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn Glu
35 40 45
Gln

<210> 58

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 58

Phe His Lys His Arg Met Pro
1 5

<210> 59

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 59

Phe His Lys His Arg Thr Leu
1 5

<210> 60

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 60

Phe His Lys His Arg Val Ser
1 5

<210> 61

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 61

Phe His Lys Pro Pro Arg Leu
1 5

<210> 62

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 62

Phe His Lys Arg Tyr Pro Pro
1 5

<210> 63

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 63

Phe His Lys Trp Pro Arg Val
1 5

<210> 64

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 64

Ile His Lys His Lys Leu Arg
1 5

<210> 65

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 65

Leu His Lys Tyr Thr Lys Ser
1 5

<210> 66

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 66

Trp His Gly Ser Trp Lys Lys

1

5

<210> 67

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 67

Trp His Lys His Pro Arg Ala

1

5

<210> 68

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 68

Trp His Lys His Gln Arg Leu

1

5

<210> 69

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 69

Trp His Lys His Thr Arg Val

1

5

<210> 70

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 70

Trp His Lys His Tyr Pro Arg
1 5

<210> 71

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 71

Trp His Lys Lys Pro Ile Pro
1 5

<210> 72

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 72

Trp His Lys Lys Thr Pro Pro
1 5

<210> 73

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 73

Trp His Lys Pro Phe Arg Phe
1 5

<210> 74

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 74

Trp His Lys Pro Arg Leu
1 5

<210> 75
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 75
Trp His Lys Pro Arg Leu Tyr
1 5

<210> 76
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 76
Trp His Lys Pro Arg Pro Thr
1 5

<210> 77
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 77
Trp His Lys Pro Trp Ile Arg
1 5

<210> 78
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 78
Trp His Lys Trp Pro Gln Arg
1 5

<210> 79
<211> 7
<212> PRT
<213> Artificial Sequence

<220>

<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 79

Trp His Lys Pro Arg Pro Arg
1 5

<210> 80

<211> 7

<212> PRT

<213> Homo sapiens

<400> 80

Gly Pro Glu Thr Leu Cys Gly
1 5

<210> 81

<211> 10

<212> PRT

<213> Homo sapiens

<400> 81

Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly
1 5 10

<210> 82

<211> 3

<212> PRT

<213> Homo sapiens

<400> 82

Phe Arg Ser
1

<210> 83

<211> 7

<212> PRT

<213> Homo sapiens

<400> 83

Pro Ser Glu Thr Leu Cys Gly
1 5

<210> 84

<211> 16

<212> PRT

<213> Homo sapiens

<400> 84

Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
1 5 10 15

<210> 85

<211> 17

<212> PRT

<213> Homo sapiens

<400> 85

22

Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp

<210> 86
 <211> 18
 <212> PRT
 <213> Homo sapiens

<400> 86
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys

<210> 87
 <211> 19
 <212> PRT
 <213> Homo sapiens

<400> 87
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His

<210> 88
 <211> 20
 <212> PRT
 <213> Homo sapiens

<400> 88
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His Gly
 20

<210> 89
 <211> 21
 <212> PRT
 <213> Homo sapiens

<400> 89
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His Gly Leu
 20

<210> 90
 <211> 22
 <212> PRT
 <213> Homo sapiens

<400> 90
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15

23

Asp Lys His Gly Leu Tyr
20

<210> 91
<211> 23
<212> PRT
<213> Homo sapiens

<400> 91
Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
1 5 10 15
Asp Lys His Gly Leu Tyr Asn
20

<210> 92
<211> 24
<212> PRT
<213> Homo sapiens

<400> 92
Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
1 5 10 15
Asp Lys His Gly Leu Tyr Asn Leu
20

<210> 93
<211> 25
<212> PRT
<213> Homo sapiens

<400> 93
Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
1 5 10 15
Asp Lys His Gly Leu Tyr Asn Leu Lys
20 25

<210> 94
<211> 26
<212> PRT
<213> Homo sapiens

<400> 94
Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
1 5 10 15
Asp Lys His Gly Leu Tyr Asn Leu Lys Gln
20 25

<210> 95
<211> 27
<212> PRT
<213> Homo sapiens

<400> 95
Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
1 5 10 15
Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys
20 25

24

<210> 96
 <211> 28
 <212> PRT
 <213> Homo sapiens

<400> 96
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys
 20 25

<210> 97
 <211> 29
 <212> PRT
 <213> Homo sapiens

<400> 97
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met
 20 25

<210> 98
 <211> 30
 <212> PRT
 <213> Homo sapiens

<400> 98
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser
 20 25 30

<210> 99
 <211> 31
 <212> PRT
 <213> Homo sapiens

<400> 99
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu
 20 25 30

<210> 100
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 100
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys
 1 5 10 15
 Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 20 25 30

<210> 101
 <211> 33
 <212> PRT

25

<213> Homo sapiens

<400> 101

Glu	Arg	Gly	Pro	Leu	Glu	His	Leu	Tyr	Ser	Leu	His	Ile	Pro	Asn	Cys
1				5					10					15	
Asp	Lys	His	Gly	Leu	Tyr	Asn	Leu	Lys	Gln	Cys	Lys	Met	Ser	Leu	Asn
			20					25					30		

Gly

<210> 102

<211> 34

<212> PRT

<213> Homo sapiens

<400> 102

Glu	Arg	Gly	Pro	Leu	Glu	His	Leu	Tyr	Ser	Leu	His	Ile	Pro	Asn	Cys
1				5					10					15	
Asp	Lys	His	Gly	Leu	Tyr	Asn	Leu	Lys	Gln	Cys	Lys	Met	Ser	Leu	Asn
			20					25					30		

Gly Gln

<210> 103

<211> 35

<212> PRT

<213> Homo sapiens

<400> 103

Glu	Arg	Gly	Pro	Leu	Glu	His	Leu	Tyr	Ser	Leu	His	Ile	Pro	Asn	Cys
1				5					10					15	
Asp	Lys	His	Gly	Leu	Tyr	Asn	Leu	Lys	Gln	Cys	Lys	Met	Ser	Leu	Asn
			20					25					30		

Gly Gln Arg
35

<210> 104

<211> 36

<212> PRT

<213> Homo sapiens

<400> 104

Glu	Arg	Gly	Pro	Leu	Glu	His	Leu	Tyr	Ser	Leu	His	Ile	Pro	Asn	Cys
1				5					10					15	
Asp	Lys	His	Gly	Leu	Tyr	Asn	Leu	Lys	Gln	Cys	Lys	Met	Ser	Leu	Asn
			20					25					30		

Gly Gln Arg Gly
35

<210> 105

<211> 37

<212> PRT

<213> Homo sapiens

<400> 105

Glu	Arg	Gly	Pro	Leu	Glu	His	Leu	Tyr	Ser	Leu	His	Ile	Pro	Asn	Cys
1				5					10					15	

26

Asp Lys His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn
 20 25 30
 Gly Gln Arg Gly Glu
 35

<210> 106
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 106
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn
 1 5 10 15

<210> 107
 <211> 14
 <212> PRT
 <213> Homo sapiens

<400> 107
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro
 1 5 10

<210> 108
 <211> 13
 <212> PRT
 <213> Homo sapiens

<400> 108
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile
 1 5 10

<210> 109
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 109
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His
 1 5 10

<210> 110
 <211> 11
 <212> PRT
 <213> Homo sapiens

<400> 110
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu
 1 5 10

<210> 111
 <211> 10
 <212> PRT
 <213> Homo sapiens

<400> 111
 Glu Arg Gly Pro Leu Glu His Leu Tyr Ser
 1 5 10

27

<210> 112
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 112
 Gly Glu Glu Ile Ser Lys Phe Tyr Leu Pro Asn Cys
 1 5 10

<210> 113
 <211> 9
 <212> PRT
 <213> Homo sapiens

<400> 113
 Pro Arg Gly Val His Ile Pro Asn Cys
 1 5

<210> 114
 <211> 12
 <212> PRT
 <213> Homo sapiens

<400> 114
 His Glu Asp Leu Tyr Ile Ile Pro Ile Pro Asn Cys
 1 5 10

<210> 115
 <211> 9
 <212> PRT
 <213> Homo sapiens

<400> 115
 Pro Arg Ala Val Tyr Leu Pro Asn Cys
 1 5

<210> 116
 <211> 14
 <212> PRT
 <213> Homo sapiens

<400> 116
 Glu Val Tyr Arg Gly Ala Gln Thr Leu Tyr Val Pro Asn Cys
 1 5 10

<210> 117
 <211> 76
 <212> PRT
 <213> Homo sapiens

<400> 117
 Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro Asn Cys Asp Lys
 1 5 10 15
 His Gly Leu Tyr Asn Leu Lys Gln Cys Lys Met Ser Leu Asn Gly Gln
 20 25 30
 Arg Gly Glu Cys Trp Cys Val Asn Pro Asn Thr Gly Lys Leu Ile Gln
 35 40 45

28

Gly Ala Pro Thr Ile Arg Gly Asp Pro Glu Cys His Leu Phe Tyr Asn
 50 55 60
 Glu Gln Gln Glu Ala Cys Gly Val His Thr Gln Arg
 65 70 75

<210> 118
 <211> 71
 <212> PRT
 <213> Homo sapiens

<400> 118
 Gly Pro Glu Thr Leu Cys Gly Ala Glu Leu Val Asp Ala Leu Gln Phe
 1 5 10 15
 Val Cys Cys Gly Ala Arg Gly Phe Tyr Phe Asn Lys Pro Thr Gly Tyr
 20 25 30
 Gly Ser Ser Ser Arg Arg Ala Pro Asn Thr Gly Ile Val Asp Glu Cys
 35 40 45
 Cys Phe Arg Ser Cys Asp Leu Arg Arg Leu Glu Met Tyr Cys Ala Pro
 50 55 60
 Leu Lys Pro Ala Lys Ser Ala
 65 70

<210> 119
 <211> 66
 <212> PRT
 <213> Homo sapiens

<400> 119
 Ala Tyr Arg Pro Ser Glu Thr Leu Cys Gly Gly Glu Leu Val Asp Thr
 1 5 10 15
 Leu Asn Phe Val Cys Gly Asp Arg Gly Phe Tyr Phe Ser Arg Pro Ser
 20 25 30
 Arg Val Ser Arg Arg Ser Arg Gly Ile Val Glu Glu Cys Phe Arg
 35 40 45
 Ser Cys Asp Leu Ala Leu Leu Glu Thr Tyr Cys Ala Thr Pro Ala Lys
 50 55 60
 Ser Glu
 65

<210> 120
 <211> 51
 <212> PRT
 <213> Porcine

<400> 120
 Phe Val Asn Gln His Leu Cys Gly Ser His Leu Val Glu Ala Leu Tyr
 1 5 10 15
 Leu Val Cys Gly Glu Arg Gly Phe Phe Tyr Thr Pro Lys Ala Gly Ile
 20 25 30
 Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu Tyr Gln Leu Glu Asn
 35 40 45
 Tyr Cys Asn
 50

<210> 121
 <211> 233
 <212> PRT
 <213> Homo sapiens

<400> 121

Ala Pro Trp Gln Cys Ala Pro Cys Ser Ala Glu Lys Leu Leu Cys Pro
 1 5 10 15
 Pro Val Ser Ala Ser Cys Ser Glu Val Thr Arg Ser Ala Gly Cys Gly
 20 25 30
 Cys Cys Pro Met Cys Ala Leu Pro Leu Gly Ala Ala Cys Gly Val Ala
 35 40 45
 Thr Ala Arg Cys Ala Arg Gly Leu Ser Cys Arg Ala Leu Pro Gly Glu
 50 55 60
 Gln Gln Pro Leu His Ala Leu Thr Arg Gly Gln Gly Ala Cys Val Gln
 65 70 75 80
 Glu Ser Asp Ala Ser Ala Pro His Ala Glu Ala Gly Ser Pro Arg Glu
 85 90 95
 Ser Pro Glu Ser Thr Glu Ile Thr Glu Glu Glu Leu Leu Asp Asn Phe
 100 105 110
 His Leu Met Ala Pro Ser Glu Glu Asp His Ser Ile Leu Trp Asp Ala
 115 120 125
 Ile Ser Thr Tyr Asp Gly Ser Lys Ala Leu His Val Thr Asn Ile Lys
 130 135 140
 Lys Trp Lys Glu Pro Cys Arg Ile Glu Leu Tyr Arg Val Val Glu Ser
 145 150 155 160
 Leu Ala Lys Ala Gln Glu Thr Ser Gly Glu Glu Ile Ser Lys Phe Tyr
 165 170 175
 Leu Pro Asn Cys Asn Lys Asn Gly Phe Tyr His Ser Arg Gln Cys Glu
 180 185 190
 Thr Ser Met Asp Gly Glu Ala Gly Leu Cys Trp Cys Val Tyr Pro Trp
 195 200 205
 Asn Gly Lys Arg Ile Pro Gly Ser Pro Glu Ile Arg Gly Asp Pro Asn
 210 215 220
 Cys Gln Ile Tyr Phe Asn Val Gln Asn
 225 230

<210> 122

<211> 289

<212> PRT

<213> Homo sapiens

<400> 122

Glu Val Leu Phe Arg Cys Pro Pro Cys Thr Pro Glu Arg Leu Ala Ala
 1 5 10 15
 Cys Gly Pro Pro Pro Val Ala Pro Pro Ala Ala Val Ala Val Ala
 20 25 30
 Gly Gly Ala Arg Met Pro Cys Ala Glu Leu Val Arg Glu Pro Gly Cys
 35 40 45
 Gly Cys Cys Ser Val Cys Ala Arg Leu Glu Gly Glu Ala Cys Gly Val
 50 55 60
 Tyr Thr Pro Arg Cys Gly Gln Gly Leu Arg Cys Tyr Pro His Pro Gly
 65 70 75 80
 Ser Glu Leu Pro Leu Gln Ala Leu Val Met Gly Glu Gly Thr Cys Glu
 85 90 95
 Lys Arg Arg Asp Ala Glu Tyr Gly Ala Ser Pro Glu Gln Val Ala Asp
 100 105 110
 Asn Gly Asp Asp His Ser Glu Gly Gly Leu Val Glu Asn His Val Asp
 115 120 125
 Ser Thr Met Asn Met Leu Gly Gly Gly Gly Ser Ala Gly Arg Lys Pro
 130 135 140
 Leu Lys Ser Gly Met Lys Glu Leu Ala Val Phe Arg Glu Lys Val Thr
 145 150 155 160

<400> 123																
Gly	Ala	Ser	Ser	Gly	Gly	Leu	Gly	Pro	Val	Val	Arg	Cys	Glu	Pro	Cys	
1				5					10					15		
Asp	Ala	Arg		Leu	Ala	Gln	Cys	Ala	Pro	Pro	Pro	Ala	Val	Cys	Ala	
			20					25					30			
Glu	Leu	Val	Arg	Glu	Pro	Gly	Cys	Gly	Cys	Cys	Leu	Thr	Cys	Ala	Leu	
		35					40					45				
Ser	Glu	Gly	Gln	Pro	Cys	Gly	Ile	Tyr	Thr	Glu	Arg	Cys	Gly	Ser	Gly	
	50					55					60					
Leu	Arg	Cys	Gln	Pro	Ser	Pro	Asp	Glu	Ala	Arg	Pro	Leu	Gln	Ala	Leu	
65					70					75					80	
Leu	Asp	Gly	Arg	Gly	Leu	Cys	Val	Asn	Ala	Ser	Ala	Val	Ser	Arg	Leu	
				85					90					95		
Arg	Ala	Tyr	Leu	Leu	Pro	Ala	Pro	Pro	Ala	Pro	Gly	Asn	Ala	Ser	Glu	
			100					105					110			
Ser	Glu	Glu	Asp	Arg	Ser	Ala	Gly	Ser	Val	Glu	Ser	Pro	Ser	Val	Ser	
		115					120					125				
Ser	Thr	His	Arg	Val	Ser	Asp	Pro	Lys	Phe	His	Pro	Leu	His	Ser	Lys	
	130					135					140					
Ile	Ile	Ile	Ile	Lys	Lys	Gly	His	Ala	Lys	Asp	Ser	Gln	Arg	Tyr	Tyr	
145					150					155					160	
Val	Asp	Tyr	Glu	Ser	Gln	Ser	Thr	Asp	Thr	Gln	Asn	Phe	Ser	Ser	Glu	
				165					170					175		
Ser	Lys	Arg	Glu	Thr	Glu	Tyr	Gly	Pro	Cys	Arg	Arg	Glu	Met	Glu	Asp	
			180					185					190			
Thr	Leu	Asn	His	Leu	Lys	Phe	Leu	Asn	Val	Leu	Ser	Pro	Arg	Gly	Val	
		195					200					205				
His	Ile	Pro	Asn	Cys	Asp	Lys	Lys	Gly	Phe	Tyr	Lys	Lys	Lys	Gln	Cys	
	210					215					220					
Arg	Pro	Ser	Lys	Gly	Arg	Lys	Arg	Gly	Phe	Cys	Trp	Cys	Val	Asp	Lys	
225				230						235					240	
Tyr	Gly	Gln	Pro	Leu	Pro	Gly	Tyr	Thr	Thr	Lys	Gly	Lys	Glu	Asp	Val	
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<211> 233

<212> PRT

<213> Homo sapiens

<400> 124

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Gly Cys Cys Ala Thr Cys Ala Leu Gly Leu Gly Met Pro Cys Gly Val
35 40 45
Tyr Thr Pro Arg Cys Gly Ser Gly Leu Arg Cys Tyr Pro Pro Arg Gly
50 55 60
Val Glu Lys Pro Leu His Thr Leu Met His Gly Gln Gly Val Cys Met
65 70 75 80
Glu Leu Ala Glu Ile Glu Ile Gln Glu Ser Leu Pro Ser Asp Lys Asp
85 90 95
Glu Gly Asp His Pro Asn Asn Ser Phe Ser Pro Cys Ser Ala His Asp
100 105 110
Arg Arg Cys Leu Gln Lys His Phe Ala Lys Ile Arg Asp Ser Thr Ser
115 120 125
Gly Gly Lys Met Val Asn Gly Ala Pro Arg Glu Asp Ala Arg Pro Val
130 135 140
Pro Gln Gly Ser Cys Gln Ser Glu Leu His Arg Ala Leu Glu Arg Leu
145 150 155 160
Ala Ala Ser Gln Ser Arg Thr His Glu Asp Leu Tyr Ile Ile Pro Ile
165 170 175
Pro Asn Cys Asp Arg Asn Gly Asn Phe His Pro Lys Gln Cys His Pro
180 185 190
Ala Leu Asp Gly Gln Arg Gly Lys Cys Trp Cys Val Asp Arg Lys Thr
195 200 205
Gly Val Lys Leu Pro Gly Gly Leu Glu Pro Lys Gly Glu Leu Asp Cys
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<211> 248

<212> PRT

<213> Homo sapiens

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20 25 30
Cys Cys Met Thr Cys Ala Leu Ala Glu Gly Gln Ser Cys Gly Val Tyr
35 40 45
Thr Glu Arg Cys Ala Gln Gly Leu Arg Cys Leu Pro Arg Asp Glu Glu
50 55 60
Lys Pro Leu His Ala Leu Leu His Gly Arg Gly Val Cys Leu Asn Glu
65 70 75 80
Lys Ser Tyr Arg Glu Gln Val Lys Ile Glu Arg Asp Ser Arg Glu His
85 90 95

32

Glu Glu Pro Thr Thr Ser Glu Met Ala Glu Glu Thr Tyr Ser Pro Lys
 100 105 110
 Ile Phe Arg Pro Lys His Thr Arg Ile Ser Glu Leu Lys Ala Glu Ala
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 Val Lys Lys Asp Arg Arg Lys Lys Leu Thr Gln Ser Phe Val Gly Gly
 130 135 140
 Ala Glu Asn Thr Ala His Pro Arg Ile Ile Ser Ala Pro Glu Met Arg
 145 150 155 160

 Gln Glu Ser Glu Gln Gly Pro Cys Arg Arg His Met Glu Ala Ser Leu
 165 170 175
 Gln Glu Leu Lys Ala Ser Pro Arg Met Val Pro Arg Ala Val Tyr Leu
 180 185 190
 Pro Asn Cys Asp Arg Lys Gly Phe Tyr Lys Arg Lys Gln Cys Lys Pro
 195 200 205
 Ser Arg Gly Arg Lys Arg Gly Ile Cys Trp Cys Val Asp Lys Tyr Gly
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 225 230 235 240
 Thr Phe Asp Ser Ser Asn Val Glu
 245

<210> 126

<211> 215

<212> PRT

<213> Homo sapiens

<400> 126

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 35 40 45
 Tyr Thr Pro Asn Cys Ala Pro Gly Leu Gln Cys His Pro Pro Lys Asp
 50 55 60
 Asp Glu Ala Pro Leu Arg Ala Leu Leu Leu Gly Arg Gly Arg Cys Leu
 65 70 75 80
 Pro Ala Arg Ala Pro Ala Val Ala Glu Glu Asn Pro Lys Glu Ser Lys
 85 90 95
 Pro Gln Ala Gly Thr Ala Arg Pro Gln Asp Val Asn Arg Arg Asp Gln
 100 105 110
 Gln Arg Asn Pro Gly Thr Ser Thr Thr Pro Ser Gln Pro Asn Ser Ala
 115 120 125
 Gly Val Gln Asp Thr Glu Met Gly Pro Cys Arg Arg His Leu Asp Ser
 130 135 140
 Val Leu Gln Gln Leu Gln Thr Glu Val Tyr Arg Gly Ala Gln Thr Leu
 145 150 155 160
 Tyr Val Pro Asn Cys Asp His Arg Gly Phe Tyr Arg Lys Arg Gln Cys
 165 170 175
 Arg Ser Ser Gln Gly Gln Arg Arg Gly Pro Cys Trp Cys Val Asp Arg
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 Met Gly Lys Ser Leu Pro Gly Ser Pro Asp Gly Asn Gly Ser Ser Ser
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 210 215

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<400> 127

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Cys Gly Pro Pro Pro Val Ala Pro Pro Ala Ala Val Ala Val Ala
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          35           40           45
Gly Cys Cys Ser Val Cys Ala Arg Leu Glu Gly Glu Ala Cys Gly Val
          50           55           60
Tyr Thr Pro Arg Cys Gly Gln Gly Leu Arg Cys Tyr Pro His Pro Gly
65           70           75           80
Ser Glu Leu Pro Leu Gln Ala Leu Val Met Gly Glu Gly Thr Cys Glu
          85           90           95
Lys Arg Arg Asp Ser Glu Tyr Gly Ala Ser Pro Glu Gln Val Ala Asp
          100          105          110
Asn Gly Asp Asp His Ser Glu Gly Leu Val Glu Asn His Val Asp
          115          120          125
Ser Thr Met Asn Met Leu Gly Gly Gly Gly Ser Ala Gly Arg Lys Pro
          130          135          140
Leu Lys Ser Gly Met Lys Glu Leu Ala Val Phe Arg Glu Lys Val Thr
          145          150          155          160
Glu Gln His Arg Gln Met Gly Lys Gly Gly Lys His His Leu Gly Leu
          165          170          175
Glu Glu Pro Lys Lys Leu Arg Pro Pro Ala Arg Thr Pro Cys Gln
          180          185          190
Gln Glu Leu Asp Gln Val Leu Glu Arg Gly Pro Glu His Leu Tyr Ser
          195          200          205
Leu His Ile Pro Asn Cys Asp Lys His Gly Leu Tyr Asn Leu Lys Gln
          210          215          220
Cys Lys Met Ser Leu Asn Gly Gln Arg Gly Glu Cys Trp Cys Val Asn
          225          230          235          240
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His Thr Gln Arg Met Gln
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<400> 128

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<211> 39

<212> PRT

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 Gly Gln Arg Gly Glu Cys Trp
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<210> 131

<211> 34

<212> PRT

<213> Homo sapiens

<400> 131

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 Pro Asp Glu Arg Gly Pro Leu Glu His Leu Tyr Ser Leu His Ile Pro
 20 25 30
 Asn Cys

<210> 132

<211> 10

<212> PRT

<213> Homo sapiens

<400> 132

Gly Pro Leu Glu His Leu Tyr Ser Leu His
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<211> 10

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 1 5 10

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<211> 10

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36

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<210> 142

<211> 13

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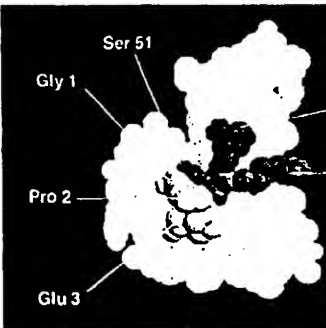
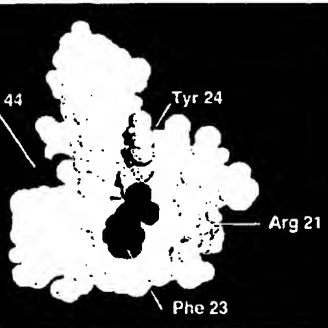
<223> NOTE: Description of Artificial Sequence =
synthetic construct

<400> 142

Cys Cys Tyr Ala Ala Pro Leu Lys Pro Ala Lys Ser Cys
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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁷ : C07K 14/47, 14/65, 7/06, A61K 38/17, 38/30, C12N 15/12</p>	<p>A3</p>	<p>(11) International Publication Number: WO 00/23469 (43) International Publication Date: 27 April 2000 (27.04.00)</p>
<p>(21) International Application Number: PCT/US99/23839 (22) International Filing Date: 14 October 1999 (14.10.99) (30) Priority Data: 60/104,528 16 October 1998 (16.10.98) US (71) Applicant: MUSC FOUNDATION FOR RESEARCH DEVELOPMENT [US/US]; 141 MUSC Complex, Suite 305, Cannon Park Place, Charleston, SC 29425 (US). (72) Inventors: ROSENZWEIG, Steven, Alan; 365 Sugar Cane Way, Mount Pleasant, SC 29464 (US). HORNEY, Mark, James; 2538 North Castle Lane, Charleston, SC 29414 (US). (74) Agents: McKEON, Tina, W. et al.; Needle & Rosenberg, P.C., Suite 1200, The Candler Building, 127 Peachtree Street, N.E., Atlanta, GA 30303-1811 (US).</p>		<p>(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> (88) Date of publication of the international search report: 24 August 2000 (24.08.00)</p>
<p>(54) Title: FRAGMENTS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN AND INSULIN-LIKE GROWTH FACTOR, AND USES THEREOF</p>		
<p style="text-align: center;">IGF-1 Binding Domains</p> <div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>IGFBP-Binding Domain</p> </div> <div style="text-align: center;">  <p>IGF-1R-Binding Domain</p> </div> </div>		
<p>(57) Abstract</p> <p>The present invention provides the portions of IGFBP and IGF peptides which account for IGF-IGFBP binding. Thus, the present invention provides an isolated IGF binding domain of an IGFBP or modifications thereof, which binds IGF with at least about the same binding affinity as the full length IGFBP. The present invention also provides an IGF antagonist that reduces binding of IGF to an IGF receptor. Further provided is a fragment of IGF or modification thereof, wherein the fragment or modification thereof binds to a binding domain of IGFBP. The present invention further provides a protein complex, comprising the IGF binding domain of IGFBP of the invention and the IGF fragment of the invention. The present invention provides methods of treating a subject with cancer and of preventing cancer in a subject, of treating a subject with a diabetic complication exacerbated by IGF and of preventing diabetic complications exacerbated by IGF comprising administering to the subject the IGF antagonist of the present invention. Also provided is a method of treating a subject with an ischemic injury or preventing an ischemic injury in a subject, comprising administering to the subject the IGF fragment or IGFBP antagonist of the invention.</p>		

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INTERNATIONAL SEARCH REPORT

International Application No

PC., US 99/23839

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/47 C07K14/65 C07K7/06 A61K38/17 A61K38/30
C12N15/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	X. QIN ET AL.,: "Structure-function analysis of the human insulin-like growth factor binding protein-4" J. BIOL. CHEM, vol. 273, no. 36, 4 September 1998 (1998-09-04), pages 23509-23516, XP000882714 the whole document, in particular Table 1 on page 23515	1,2,4,5, 8,12,15, 45-47
Y	---	3,6,7, 9-11
	---	---



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

Date of the actual completion of the international search

6 March 2000

Date of mailing of the international search report

09.06.00

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Julia, P

INTERNATIONAL SEARCH REPORT

International Application No

PC./US 99/23839

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EM SPENCER AND K CHAN: "A 3-dimensional model for the insulin-like growth factor binding proteins (IGFBPs); supporting evidence using the structural determinants of the IGF binding site on IGFBP-3" PROG. GROWTH FACTOR RES., vol. 6, no. 2-4, 1995, pages 209-214, XP000881394 abstract, pages 213-214 and figure 5 ---	1,2,4,5, 7,12,15, 45-47
X	BE FORBES ET AL.,: "Localization of an insulin-like growth factor (IGF) binding site of bovine IGF binding protein-2 using disulfide mapping and deletion mutation analysis of the C-terminal domain" J. BIOL. CHEM., vol. 273, no. 8, 20 February 1998 (1998-02-20), pages 4647-4652, XP000882716 whole document, in particular figures 4-6 and table II ---	1-5,12, 15,45-47
X	PJ HO AND RC BAXTER: "Characterization of truncated insulin-like growth factor-binding protein-2 in human milk" ENDOCRINOLOGY, vol. 138, no. 9, September 1997 (1997-09), pages 3811-3818, XP000881395 the whole document ---	1-5,11, 12,15
X	JF WANG ET AL.,: "Isolation of a biologically active fragment from the carboxy terminus of the fetal rat binding protein for insulin-like growth factors" BIOCHEM. BIOPHYS. RES. COMMUN., vol. 157, no. 2, 15 December 1988 (1988-12-15), pages 718-726, XP000881403 the whole document ---	1,2,5, 11,12,15
X	WO 92 12243 A (CHIRON CORPORATION) 23 July 1992 (1992-07-23) abstract, page 3 lines 18-29, page 6 lines 31-36, page 8 lines 26-30, page 9 lines 4-14, page 50 and claims 1 and 3 ---	1,2,5, 10,12, 15, 29-42, 45-47
A	WO 94 22466 A (SYNERGEN, INC.) 13 October 1994 (1994-10-13) the whole document in particular page 9 lines 1-2 ---	29-42

	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PC., US 99/23839

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SL. DROP ET AL.,: "Structural aspects of the IGFBP family" GROWTH REGUL., vol. 2, no. 2, June 1992 (1992-06), pages 69-79, XP000881414 the whole document	1,2,4,5, 8
Y	the whole document, in particular abstract and page 73 -----	3,6,7, 9-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/23839

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 29-42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11, 15-17, 36-42, 45-47 (complete) and 12-13, 29-35 (partial)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-11, 15-17, 36-42, 45-47 (complete) and 12-13, 29-35 (partial)

concerned with an isolated peptide comprising an insulin-like growth factor (IGF) binding domain of an IGF binding protein (IGFBP) and which binds IGF with at least about the same binding affinity as the full-length IGFBP; an IGF antagonist comprising a peptide of 250 or fewer amino acids, wherein the peptide can displace bound IGFBP from IGF and wherein the peptide reduces binding of IGF to an IGF receptor and wherein said peptide comprises an IGF binding domain of an IGFBP; methods of therapeutical treatment using the isolated peptide comprising an IGFBP binding domain or using the IGF antagonist; recombinant production of the isolated peptide (nucleic acid, vector and host cell).

2. Claims: 14 (complete) and 12-13, 29-35 (partial)

concerned with an IGF antagonist comprising a peptide of 250 or fewer amino acids, wherein the peptide can displace bound IGFBP from IGF and wherein the peptide reduces binding of IGF to an IGF receptor and wherein said peptide does not comprise an IGF binding domain of an IGFBP.

3. Claims: 18-23, 43-44

concerned with a fragment of IGF, wherein the fragment binds to a binding domain on IGFBP; methods of therapeutical treatment using this IGF fragments.

4. Claims: 24-28

concerned with a protein complex, comprising (i) an isolated peptide comprising an IGF binding domain of an IGFBP, which binds IGF with at least about the same binding affinity as the full-length IGFBP and (ii) a fragment of IGF, wherein the fragment binds to a binding domain on IGFBP

5. Claims: 48-49

concerned with an isolated IGF-1, wherein one residue of the IGF-1 is biotinylated and wherein the biotinylated IGF-1 has affinity for IGFBP-2 of about 300 to 50 pM in a competition binding assay.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC, US 99/23839

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9212243 A	23-07-1992	CA 2100083 A EP 0556344 A PT 99995 A,B	09-07-1992 25-08-1993 29-01-1993
WO 9422466 A	13-10-1994	AU 6626794 A CA 2160154 A CN 1134111 A EP 0708655 A FI 954805 A JP 8508296 T	24-10-1994 13-10-1994 23-10-1996 01-05-1996 15-11-1995 03-09-1996

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